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MICROBIOLOGY

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MICROBIOLOGY

A TEXT-BOOK OF
MICROÖRGANISMS GENERAL AND APPLIED

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WITH 200 ILLUSTRATIONS

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INTRODUCTION TO THE THIRD EDITION

The kindly reception of Microbiology, which has been progressive, makes a revision a pleasurable task.

There has been little need of change in the basic facts presented, but there is always room for a clarification of thought and improvement in arrangement. As time has passed it has been found desirable, also, to emphasize and extend some of the chapters.

Teaching has demonstrated that, in most instances, the chapters dealing with biological products follow more naturally and logically the chapter on immunity. Since the chapters on diseases are more of a reference character, they have been placed at the end.

The war has made more prominent food contamination, preservation and decomposition. For this reason all chapters considering food have been brought together in a single division and greater attention has been given the subject by rewriting, insertions and enlarging the scope. Dairy microbiology has not been included in the division of food because it has such a distinctive field of its own.

The editor has a deep feeling of indebtedness to the contributors who have been so kindly disposed, ready and helpful in this revision, and to Miss Marion F. Dondale, for her immeasurable assistance.

Charles E. Marshall, Editor.

Amherst, Massachusetts.

INTRODUCTION TO THE SECOND EDITION

The continued and growing demand for "*Microbiology*" has caused the contributors to undertake a thorough revision. In this they have been guided by the recent developments in this branch of science, and also by a desire to adjust and rearrange in the light of constructive suggestions and criticisms.

The primary purpose of this text-book is to place in the hands of college students an elementary technical treatise of the subject matter included. No effort has been made to review or cite literature, for to do either would expand the volume beyond useful limits. To provide an introductory text-book mainly for recitations, or for a supplement to lecture or laboratory courses, is about all that can be satisfactorily comprehended in a single project.

The cytological aspect of microbiology has seemed to us to deserve some emphasis, for it has become quite definite and has been suggestively indicating much of real value in connection with the active life processes of the cell and microbic activities in agriculture, medicine and wherever microbiology is applicable.

The significance of "Intestinal Microbiology" has required a short chapter for its proper presentation.

It has also been found desirable to treat the microbial diseases of insects, a growing subject, in a distinct chapter.

The study of microörganisms flounders in a fog of unsettled ideas for a proper designation. Whether it should be called Protistology, Microbiology, Bacteriology, Mycology, or something else must be left for the future to determine.

CHARLES E. MARSHALL, EDITOR.

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INTRODUCTION TO THE FIRST EDITION

By a process of adaptation and growth, the branch of science commonly recognized as "*Bacteriology*" has for many years included, besides the bacterial forms, those microörganisms yielding to the same laboratory methods of study and investigation. This is a policy or purpose instituted by Pasteur. It is also the result of investigations and added knowledge, more definite arrangements of available facts, and the highly specialized training required for the work. In short, technic together with the economic relations of the subject-matter has no little influence in placing limitations. In the light of such circumstances, it appears more pertinent to designate this text-book as "*Microbiology*," perhaps not the best term, but one much in accord with French usage.

Agriculture, Domestic Science and certain other courses in scientific schools and colleges call for the treatment of the subject in such a manner as to make it basic to the interpretation of such subjects as air impurities, water supplies, sewage disposal, soils, dairying, fermentation industries, food preservation and decomposition, manufacture of biological products, transmission of disease, susceptibility and immunity, sanitation, and control of infectious or contagious diseases. A strong effort has been made to provide the fundamental and guiding principles of the subject and to show just how these principles fit into the subjects of a more or less strictly professional or practical nature. Here the instructional work of the microbiologist stops in most educational institutions and the instruction of the practical or professional man begins.

Because of the extreme massiveness and diversity of the subjects, Agriculture and Domestic Science and Industrial Vocations in general, a comprehensive consideration of the subject is demanded. Elimination of many features not only becomes difficult but really precarious, because so many avenues are open to the student that pertinency cannot always be foreseen or determined. It is well to remember, too, that such aggregate subjects as Agriculture and Domestic Science, unlike Engineering and Medicine, because of their youth, have not developed to that stage in their educational history where practice and the science upon which practice should be founded are amalgamated. The practical man in Agriculture, and Applied Sciences generally, too frequently is so extremely traditional in his practice that he utterly fails to separate the true from the false, or, in other words, does not exercise his discriminative powers at all, but depends entirely upon so-called haphazard methods and self-willed processes. This factor operates against the proper development and logical study of any branch of science in its relation to the farmer, or manufacturer.

The plan of a text-book in Microbiology which seeks to furnish basic principles, to train the mind in logical development and adjustment, and to prepare the student to undertake an intelligent study of strictly professional or practical subjects, must assume a definite and systematic arrangement. With this in mind, the text has been divided into three distinct parts: *Morphological and Cultural*, or that which deals with forms and methods of handling; *Physiological*, or that which deals strictly with functions, the key to the applied; *Applied*, or that which reaches into the application of the facts developed to the problems met in the study of professional or practical affairs.

In a text-book, *the product of several hands*, there is the most serious difficulty in obtaining unity of thought and expression without repetition; besides, that very conspicuous weakness of emphasizing some features unduly while other features of importance are scarcely mentioned, confronts us. A most earnest attempt has been made to overcome these faults as far as possible, but a complete mastery of them cannot be expected in the first product. However, what is lacked in unity and continuity of expression and in balance we sincerely hope will be made up, in part at least, by the selection and the value of the material contributed.

Laboratory features of microbiology have been eliminated wherever it has been practicable. Should any demonstrations be added or needed, we have felt that they may be easily supplied by the instructor, who, of course, will be governed by local facilities and conditions. Although no space has been given to laboratory exercises, it should not be gathered that the authors of this book are any the less earnest in urging a well-organized laboratory course to supplement the general instruction as an essential factor to a working appreciation of the subject.

In matters of spelling, new words, and phrases, conservatism has controlled. Arbitrary decisions and selections have been forced in several instances to secure clearness, consistency and definiteness. It is painfully evident to anyone attempting to bring system out of the confusion and chaos existing in many fields of microbiological action that some rearrangement ought to be undertaken. As usual, however, this will be very slow on account of the many almost insurmountable difficulties.

We need and invite helpful suggestions and criticisms at all times, for a valuable text-book of the nature of this is one of slow growth and development and not of "sport evolution." The editor is certain that each contributor will welcome suggestions and, further, will be in far better position to judge his own contribution after the material appears in book form and has been submitted to students for which it is designed.

No one better than the editor realizes fully the sympathetic part played by the contributors. If any merit attaches to this book as it finds its place in microbiological instruction, *such merit* should be recognized as due the contributors whose unselfish aims have made it possible.

CHARLES E. MARSHALL, EDITOR.

AMHERST, MASSACHUSETTS.

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HISTORY OF MICROBIOLOGY*

Geronimo Fracastorio, of Verona, was born in 1484, studied medicine in Padua, and published a work in Venice in 1546, which contained the first statement of the true nature of contagion, infection, or disease organisms, and of the modes of transmission of infectious disease. He divided diseases into those which infect by immediate contact, through intermediate agents, and at a distance through the air. Organisms which cause disease, called *Seminaria contagionum*, he supposed to be of the nature of viscous or glutinous matter, similar to the colloidal states of substances described by modern physical chemists. These particles, too small to be seen, were capable of reproduction in appropriate media, and became pathogenic through the action of animal heat. Thus Fracastorius, in the middle of the sixteenth century, gave us an outline of morbid processes in terms of microbiology.

Athanasius Kircher, in 1659, demonstrated the presence of "minute living worms in putrid meat, milk, vinegar, etc.;" but he did not describe their form and character, and it is doubtful whether he ever saw microörganisms.

In the year 1683 Antonius van Leeuwenhoek, a Dutch naturalist and a maker of lenses, communicated to the English Royal Society the results of observations which he had made with a simple microscope of his own construction, magnifying from 100 to 150 times. He found in water, saliva, dental tartar, etc., what he termed "animalcula." He described what he saw, and by his drawings showed both rod-like and spiral forms, both of which, he said, had motility. In all probability, the two species he saw were those now recognized as *Bacillus buccalis maximus* and *Spirillum sputigenum*. Leeuwenhoek's observations were purely objective and in striking contrast with the speculative views of M. A. Plenciz, a Viennese physician, who in 1762 published a germ theory of infectious diseases. Plenciz maintained that there was a special organism by which each infectious disease was produced, that microörganisms were capable of reproduction outside of the body, and that they might be conveyed from place to place by the air.

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* Prepared by F. C. Harrison.

The important rôle that the compound microscope has played in microbiology calls for something regarding the invention of this instrument—an invention which antedates Leeuwenhoek's discovery by nearly 100 years.

The first compound microscope was made by Hans Jansen and his son Zaccharias, in 1590, at Middelburg, in Holland. The instrument was composed of two lenses mounted in tubes of iron; a representation of it, made from the original and still kept at Middelburg, is shown in Fig. 1. From that date the microscope gradually improved. In 1844 the immersion lens was introduced by Dolland. In 1870 Abbé brought out the substage condenser, which still bears his name. Apochromatic lenses and many minor improvements were introduced by the firm of Zeiss about 1880.

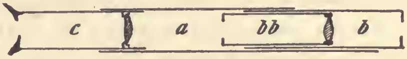

FIG. 1.—Longitudinal section of a compound microscope made by Zaccharias Jansen (1590). *a*, Microscope tube; *b*, objective tube; *c*, ocular.

In 1786 O. F. Müller (a Dane) first attempted to classify, according to the Linnean system, the various organisms previously discovered, and characterized four or five genera—among them, the genus *Vibrio*, in which, under the terms *bacillus*, *lineola*, and *spirillum*, we recognize forms that correspond with our "*bacteria*."

From the middle of the eighteenth century until well on into the nineteenth, the history of bacteriology is largely the story of a controversy between those who believed that minute living organisms, such as those above referred to, were produced from inanimate substances, and that their formation was *spontaneous*. Philosophers, poets, and common people of the most enlightened nations accepted this doctrine down to the eighteenth century. The hypothesis regarding this formation was known as that of "spontaneous generation," "heterogenesis," and "abiogenesis." The opponents of this theory denied the possibility of a transition from a lifeless to a living condition, and contended that all life came from preëxisting life—a theory aphoristically summed up in the phrase "*omne vivum ex vivo*." Such was the doctrine of Biogenesis—life only from life.

In 1668, Francisco Redi, an Italian, distinguished alike as scholar, poet, physician, and naturalist, expressed the idea that life in matter is always produced through the agency of preëxisting living matter; but the beginnings of the real controversy date from the publication of Needham's experiments in 1745. The English divine boiled some meat extract in a flask, made the flask air-tight, and left it for some days. When the flask was opened, he found in it what he termed "infusoria." He naturally concluded that all life had been killed by boiling; and, as the entrance of fresh life from the outside was prevented by the closing of the flask, he considered that the living infusoria must have originated spontaneously from the inanimate constituents of the broth.

Twenty years later Abbé Spallanzani alleged that the development of the infusoria "in an infusion maintained at boiling-point for three-quarters of an hour was possible only, provided air, which had not been previously exposed to the influence of fire, had been admitted." Objections were made to these experiments and the controversy went merrily on. Gradually experimental evidence accumulated—resulting largely from the work of Franz Schulze, and the discovery by Schroeder and Dusch in 1853, that putrescible fluids will not decay after boiling, if protected from the bacteria of the air by means of a cotton-wool filter or plug; and the epoch-making experiments of Pasteur in 1860, with the now well-known Pasteur flask, showed conclusively that the hypothesis of spontaneous generation, or abiogenesis, could not be proved.

Liebig, the celebrated German chemist, strenuously opposed the theories of Pasteur; his authority and the brilliancy of his expositions influenced the scientific world during the period 1840–60. To Liebig, fermentation was a purely chemical phenomenon unassociated with any vital process; and he treated Pasteur's results with disdain. "Those who pretend to explain the putrefaction of animal substance by the presence of microörganisms," he wrote, "reason very much like a child who would explain the rapidity of the Rhine by attributing it to the violent motions imparted to it in the direction of Bingen by the numerous wheels of the mills of Mayence." Again and again Liebig formally denied the correctness of Pasteur's assertions; finally Pasteur challenged him to appear before the Academic Commission to which they would submit their respective results. Liebig, however, did not accept the challenge; the victory was with the French savant.

In 1841 Fuchs investigated some blue and yellow milk. He examined it with the microscope and discovered the presence of organisms. He succeeded in cultivating the "blue milk" microbe in mallow slime, and re-developed the blue color in milk by introducing some of his culture. The organisms obtained were sent to Ehrenberg, who named them *Bacterium syncyaneum*, now known as *B. cyanogenus*, *Ps. syncyanea* and *B. synxanthus*, a name which is still retained in the literature.

Since 1860 the master mind of Louis Pasteur has dominated the realm of microbiology. His epoch-making discoveries were largely due to his intuitive vision, his skill in device and in the adaptation of means to ends, his prodigious industry, and the enthusiasm and love with which he inspired his associates. Trained as a chemist, his first appointment was to a professorship of chemistry, and his earliest research dealt with problems in molecular chemistry and physics. On his being elected Dean of the Faculty of Sciences at Lille, he commenced to study fermentation. His work in this field was soon followed by important results: the discovery of the organisms which produce lactic and butyric fermentation, and of anaerobic life, or life which flourishes without free oxygen. He devised an improved method of making vinegar, and demonstrated the presence of the *acetic* organism which he named *Mycoderma aceti*. Later he studied the diseases of wine, and discovered that bitterness or greasiness was due to a special ferment, and suggested the heating of wines in closed bottles to a temperature of 60°, in order to kill the injurious microörganisms. This process, since called pasteurization, is now largely used, and makes it possible for manufacturers and merchants to keep and export wine without losing its flavor or bouquet. It is interesting in this connection to note that a French confectioner named Appert published, in 1811, his method of preserving fruits, vegetables, and liquors by heating and sealing, and hence may be looked upon as the founder of the packing and canning industry.

In 1864–65 the silk districts of that region of France, known as the Midi, suffered such serious losses that the yield of cocoons fell from twenty-six million kilograms to four million, which entailed a loss of twenty million dollars and caused widespread distress and poverty. An epidemic had broken out among the silk-worms—the dread disease known as Pébrine. Pasteur was induced to make an investigation as to the best means of combating the epidemic; and, after several years of study, he found the organism causing the disease, suggested remedies, and brought back wealth to the ruined communities, but at the cost to himself of impaired health and partial paralysis.

Pasteur's results were very suggestive; and one outcome of his work was that between 1870 and 1880 several important discoveries were made by other investigators. Prior to the dates mentioned, the mortality from blood poisoning, gangrene, and other infections following operations was extremely high. Surgeons regarded such a result as inevitable, and many agreed with the saying of Velpeau, that "the prick of a pin is the open door to death;" but, in 1860, Joseph Lister, an Edinburgh surgeon, began to study the possible rôle of microbes in the infection of wounds. By sterilizing his instruments, sponges, ligatures, etc., and using antiseptics, he was able to obtain such a high percentage of recoveries that in two years he saved thirty-four patients out of forty—a percentage unheard of up to that time. Hence the origin of the antiseptic and aseptic methods of surgery is traceable to Lister's efforts. Lister's methods, suggested by the ideas of Pasteur, have rendered possible the marvelous surgery of the present day, banished hospital gangrene, and robbed confinement of its terrors.

To Lister must also be given the honor of devising the first practical way of obtaining a pure culture of bacteria by means of high dilutions. By using this method, Lister obtained some idea of the different fermentations of milk, such as souring, curdling, etc. He also confirmed the conclusion of Robert Hall (1874), that milk could be obtained from the animal in a sterile condition, thus proving that the souring of milk was caused by organisms from some external source.

In 1872, F. Cohn's System of Classification, based on morphological characters, appeared. He distinguished six genera—micrococcus, bacterium, bacillus, vibrio, spirillum, and spirochæte; four years later this investigator made the important discovery of endospores (spores formed within cells), and noticed that organisms in this state were more resistant to heat than the rods from which they were derived. This fact was observed in the well-known "*hay bacillus.*"

In 1871, Weigert succeeded in staining bacteria with picro-carmine; but it was not until 1876 that he used the aniline colors, or dyes, for this purpose, and thus opened up a new field which was exploited with such beautiful results by Ehrlich, Koch, Gram, and others. The staining of microörganisms rendered it possible to obtain pictures of them by photographic methods; the art of photomicrography developed thus rapidly.

In 1879, Miquel discovered bacteria which grew or developed at temperatures between 65°* and 75°. He isolated them first from the waters of the Seine, and subsequently from dust, manure, and other substances. Later researches have shown that these thermophilic organisms play important rôles in various fermentations.

The ninth decade of the last century was prolific in important bacteriological events. Discovery followed discovery in rapid succession. In 1880, Laveran, a French military surgeon, discovered the protozoön of malaria; in 1881 Robert Koch introduced the poured gelatin and agar plate, which made it possible to obtain pure cultures without difficulty. Investigators were quick to take advantage of this method and notable results followed. Eberth and Gaffky discovered the bacillus of typhoid fever, and succeeded in growing it in culture media. In 1882, Loeffler and Schütz discovered the bacterium which causes glanders; and in the following year Koch isolated the vibrio of Asiatic cholera from the intestines of cholera patients. In 1883 Klebs described the diphtheria bacterium; and, in 1884, Loeffler grew the organism in pure culture.

In 1884, Koch published his results on the etiology of tuberculosis, in a paper which will remain as a classical masterpiece of bacteriological research, owing to the difficulty of the task and the thoroughness of the work. Not only did Koch show the tubercle bacterium by appropriate staining methods, but he succeeded in obtaining pure cultures of it and in producing tuberculosis by inoculation with his isolated cultures.

In 1885, Nicolaier observed the tetanus bacillus in pus produced by inoculating mice and rabbits with soil; later, in 1889, Kitasato isolated this organism, and showed that the cause of the failure in earlier attempts to isolate it were due to the fact that it could grow only in the absence of free oxygen. The specific infecting agents in pneumonia were discovered by Friedlander and Fraenkel about this time, as were also several organisms associated with inflammation and suppuration, such as the *Streptococcus pyogenes* and the *Staphylococcus pyogenes*, discovered by Rosenbach, and the green pus germ (*Pseudomonas pyocyanea*) by Gessard.

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*All temperatures are stated in Centigrade scale, unless otherwise indicated.

While these discoveries were taking place, largely in Germany, Pasteur had been engrossed with his prophylactic studies. In 1880, he discovered a method of vaccination against fowl cholera; and in 1881 he published his method of vaccination against anthrax. On a farm at Pouilly le Fort, sixty sheep were placed at Pasteur's disposal; ten of these received no treatment, and twenty-five were vaccinated. Some days afterward the latter were inoculated with virulent anthrax, and also twenty-five which had received no vaccine. The twenty-five non-vaccinated sheep died, and the twenty-five vaccinated ones remained healthy and in the same state as the ten control animals. This convincing experiment was followed by others; and, in the twenty-five years immediately following the introduction of the method, more than ten million animals were vaccinated in France alone, with excellent results. In 1885, as the result of much animal experimentation, Pasteur related to the Academy of Sciences his discovery of a method of vaccination against rabies, or hydrophobia; and six months after the successful treatment of the first case, 350 persons bitten by rabid dogs were vaccinated. An institute for the preparation of vaccines was built by public subscription and named the Pasteur Institute; and since that date more than thirty similar establishments have been founded in different parts of the world.

This eighth decade, so pregnant with discoveries of the utmost importance to medicine and surgery, was also notable for its discoveries in agricultural bacteriology. The honor of having been the first to work out the causal relation between a specific microbe and a plant disease belongs to Burrill, who discovered the organism of Fire or Pear Blight; and in 1883 to 1888 Wakker discovered the bacillus which produces the "yellows" of the hyacinth, a disease of considerable economic importance in Holland. To Beyerinck, Hellriegel, and Wilfarth we owe our earlier knowledge of the development and morphology of the nitrogen-fixing organism which produces the nodules or tubercles on the roots of legumes. In 1888 Winogradsky isolated from soils nitrifying microbes which grew in a medium devoid of all traces of organic matter. During this period, Hansen's investigations along the line of the fermentation industry were most important. He devised methods for securing pure cultures of yeasts starting from a *single cell*, showed that yeasts produced diseases in beer, and established the method of identifying yeasts by observing their microscopic appearance, the formation of ascospores, and the production of films.

The tenth decade of the nineteenth century was almost as prolific in discovery as the ninth. In 1890 Behring discovered the antitoxin for diphtheria, as a result of the pioneer work on toxins by Roux and Yersin. Five years later, this serum came into general use as a curative agent; and the efficiency of the treatment is shown by a comparison of the death rate from diphtheria before and after the introduction of the antitoxin. The average annual death rate from diphtheria in eight large cities, during the period 1885–94, was 9.74 per 10,000 of the population before the use of antitoxin; and during the antitoxin period of 1895–1904 it was 4.29.

The subsequent researches on the constitution of toxins and antitoxins by Ehrlich, Metchnikoff, Madsen, and others have been productive of a better understanding of the problems of immunity.

In 1892 Pfeiffer discovered the organism of influenza or grippe; and in 1894 Yersin and Kitasato independently discovered the bacterium of bubonic plague.

The now well-known serum diagnosis of typhoid fever, whereby living and motile typhoid bacilli are clumped and lose their motility when placed in the diluted serum of a patient suffering from the fever, was due to the work of Gruber and Durham, and the exploitation of the method by Widal dates from 1896.

In 1898, Shiga discovered the bacterium of dysentery, and the possible cause of pleuro-pneumonia in cattle was found by Nocard. This latter organism was so minute as to be at the extreme limit of microscopic definition, and suggested that other well-known diseases, such as foot-and-mouth disease, are probably caused by ultra-microscopic organisms.

This year, Ronald Ross worked out the relation between man, the mosquito, and the malarial parasite—a discovery which at once suggested the best means of controlling the disease.

In 1905, Schaudinn definitely established the causal agent of syphilis, a spirochæte-shaped organism, which he named *Treponema pallidum*, and which had escaped earlier discovery on account of its being refractory to the ordinary staining methods.

In the last decade, our knowledge of certain communicable diseases has been extended considerably. Preventive and prophylactic measures have been studied extensively and carried out on a scale never before contemplated, and probably made possible only by war conditions. A few of these may be mentioned as examples of the progress made:—the Dakin-Carrel treatment of septic wounds, the immunization of troops against typhoid, tetanus and pneumonia; the increasing use, improvement in manufacture and efficacy of protective and curative sera and vaccines; the importance of the carrier in many infections, and the means whereby he is dealt with, as instanced in the case of infection with the meningococcus; the discovery of filtrable viruses as, to quote the most recent (1919), the inciting agent of mumps.

No one can deny that the progress of microbiology in the last fifty years has been wonderful, and in the last few years extraordinary, but much still remains unknown and new problems appear from time to time. The etiology of certain diseases yet remain undiscovered. The cause of the disease known as influenza which carried off so many in the fall of 1918 remains as yet unknown although some reports of alleged discoveries have been made. Trench fever is another example of a problem suddenly appearing and necessitating instant solution.

"In the last few years a group of pleomorphic organisms have been discovered, which are associated with typhus, Rocky Mountain fever and trench fever. These organisms are carried by insects but have not yet been cultivated."

So also with other fields of research. Great progress has been made in water and food microbiology; more attention is being paid to parasitology; soil organisms and especially soil protozoa are receiving more study and our technique has advanced with great strides.

In short the work of the microbiologist has become of increasing interest and importance in all lines of work.

The record of past achievement is an inspiration; and the knowledge that each discovery is the result of persistent and concentrated effort, may give us of the present day firmer faith and greater strength for work in the broad and inviting field outlined in this text book.

PART I

THE MORPHOLOGY AND CULTURE OF MICRO-ORGANISMS

GENERAL*

Microbiology is concerned with organisms which range between well defined plant life on the one hand, and well defined animal life on the other. These living forms are in the main *unicellular* in structure. A gradation exists from the plant world into this microbe-world and also from the animal world. No sharp lines can be established because Nature seems to blend from one type into another leaving no particularly characteristic barrier, although man, for his own convenience, strives to construct Nature with very definite lines of demarcation. Haeckel was so impressed with the organisms which lie between the animal and plant world that he found it undesirable to attempt to classify them in the one or the other kingdom. Accordingly, he believed it of sufficient importance to give a specific name, *Protista*, to the microörganisms included in this specific kingdom. This relationship is clearly set forth by an illustration furnished by Minchin† (Fig. 2).

Morphology has been paramount in classification in the past, yet, at first, bacteria were called animals and later plants. With the advancement and importance of physiology, it becomes necessary to consider physical, chemical, nutritive or digestive and general physiological processes along with morphological characters. When these are considered there is a marked resemblance of microörganisms, even molds and yeasts, to animal life. Assignment to either animal or plant life is precarious and unnecessary, for in making such an attempt the scientist really does nothing more than prescribe for Nature restrictions rather than follow Nature as she exists.

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* Editor.

† Minchin, E. A : An Introduction to the Study of the Protozoa.

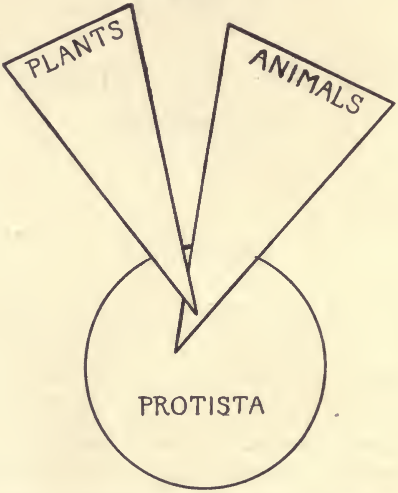

Fig. 2.—Graphic representation of the relation of the animal and vegetable kingdoms to the kingdom of Protista (*Protistenreich*). The Protozoa are represented by the portion of the triangle representing the animal kingdom which lies within the circle representing the Protista. (*After Minchin.*)

From the organization of microbiology by Pasteur, the *technic* of the subject together with, in large part as well, its *economic bearing* seems to be the applied determining factor in bounding the field. The subject of microbiology is following at present the course of all scientific branches—it is undergoing division for purposes of intensification demanded by practice and by the limitations of man's capacity.

OUTLINE OF PLANT GROUPS*

The following is a diagram of plant groups, showing one scheme of placing the bacteria, yeasts, and molds in relation to other groups. Only a few of the sub-groups can be shown in such a scheme.

- Plants
  - Schizophyta (fission-plants)
    - Schizomycetes (fission-fungi), bacteria.
    - Schizophyceæ (fission-algæ), blue-green algæ.
  - Thallophyta
    - Algæ
      - Chlorophyceæ—green algæ.
      - Phaeophyceæ—brown algæ.
      - Rhodophyceæ—red algæ.
    - Characeæ.
    - Fungi
      - Myxomycetes.
      - Actinomycetes
      - Phycomycetes
        - Chytridineæ.
        - Zygomycetes (*Mucors*).
        - Oomycetes
          - Saprolegniaceæ. (water fungi).
          - Peronosporaceæ. (downy mildews).
      - Ascomycetes
        - Hemiasci (Monascus).
        - Euasci
          - Protoascineæ (Saccharomyces, Yeasts).
          - Protodiscineæ.
          - Discomycetes.
          - Plectascineæ (Aspergillus, certain Penicillia)
          - Pyrenomycetineæ.
      - Imperfect Fungi, Conidia only
        - Penicillium, Fusarium, Alternaria,†
        - Oidium, Cladosporium, and others.
      - Basidiomycetes
        - Rusts.
        - Smuts.
        - Mushrooms.
  - Bryophyta (mosses and liverworts).
  - Pteridophyta (ferns, etc.)
  - Spermatophyta (seed plants).

† Ascomycetous species occur among these genera but such species are rarely met in bacteriological work; many of the common species of Aspergillus lack the ascigerous form, hence are classified by their conidial forms only.

OUTLINE OF PROTOZOAL GROUPS†

"AN OUTLINE CLASSIFICATION OF THE PROTOZOA," embracing only parasitic and more especially the forms pathogenic for man and domestic animals. For discussion of *classification* see p. 133.

- Protozoa
  - Rhizopoda
    - Entamœba
      - *Entamœba buccalis*
      - *Entamœba coli*
      - *Entamœba histolytica*
      - *Entamœba meleagridis*
    - Plasmodiophora
      - *Plasmodiophora brassicæ.*

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* Charles Thom.

† J. L. Todd.

- Protozoa
  - Flagellata
    - Leishmania
      - *Leishmania donovani*
      - *Leishmania tropica*
      - *Leishmania infantum*
    - Crithidia
    - Trypanosoma
      - *Trypanosoma gambiense*
      - *Trypanosoma rhodesiense*
      - *Trypanosoma cruzi*
      - *Trypanosoma brucei*
      - *Trypanosoma equinum*
      - *Trypanosoma evansi*
      - *Trypanosoma lewisi*
      - *Trypanosoma equiperdum*
    - Trypanoplasma
    - Cercomonas
    - Trichomonas
      - *Trichomonas intestinalis*
      - *Trichomonas vaginalis*
    - Monas
    - Plagiomonas
    - Lamblia
      - *Lamblia intestinalis*
  - Sporozoa
    - Gregarina
    - Coccidium
      - *Eimeria cuniculi* (*Coccidium stiedæ*)
      - *Eimeria avium*
    - Hæmosporidia
      - Plasmodium
        - *Plasmodium vivax*
        - *Plasmodium malariæ*
        - *Plasmodium falciparum*
      - Proteosoma
      - Hæmoproteus
      - Hæmogregarina
      - Hepatozoön
      - Babesia
        - *Babesia bovis* (*bigemina*)
        - *Babesia canis*
        - *Babesia parva*
        - *Bartonella*
        - *Anaplasma*
    - Sarcosporidia
      - Sarcocystis
        - *Sarcocystis miescheriana*
    - Haplosporidia
      - Rhinosporidium
        - *Rhinosporidium kinealyi*
    - Myxosporidia
      - Myxobolus
        - *Myxobolus pfeifferi*
    - Microsporidia
      - Nosema
        - *Nosema bombycis*
  - Infusoria
    - Balantidium
      - *Balantidium coli*
- Parasites of uncertain position
  - Toxoplasma
  - Histoplasma
  - Chlamydozoa
  - Rickettsia
  - Ultramicroscopic viruses
  - Spirochæta
    - *Spirochæta recurrentis*
    - *Spirochæta vincenti*
    - *Spirochæta gallinarum*
  - Treponema
    - *Treponema pallidum*
    - *Treponema pertenue*

CHAPTER I*

ELEMENTS OF MICROBIAL CYTOLOGY

CELLS AND ENERGIDS

The microörganisms are confined to cells, such as algæ, molds, bacteria, yeasts, and protozoa, or cytoplasmic masses with a nucleus associated with each (Fig. 3). Some are, however, made up of rows of cells, such as threads of *Cladothrix*, occasionally capable of branching out, like the mycelium of a mold (Fig. 4, *A*). There are also some cells which have a special structure. In each cell are enclosed several nuclei. If certain amœbæ are examined, for example, *Pelomyxa palustris* (Fig. 4, *B*), inside of what appears to be a cell there are found many nuclei. Such cells have not the anatomical value of true cells, but seem to represent as many cells as there are nuclei. Each of these nuclei with the cytoplasm which surrounds it, equivalent to a cell, may be called specifically an *energid*. Some algæ and fungi are made up of threads of cells enclosing several nuclei; each cell included in a thread consequently represents a group of organized elements, the union of several energids in the same anatomical unit (Fig. 4, *A*).

STRUCTURE OF THE CELL

A typical cell is constituted of three essential elements: the nucleus; the cytoplasm; and the cell-membrane.

The general characteristics of these three elements, and, following this, the study of cell reproduction, may now be systematically presented.

THE NUCLEAR STRUCTURE.—*General Structure of the Nucleus.*—The nucleus frequently takes in microörganisms the typical form which it assumes in the higher organisms, namely, that of a spherical vesicle limited by a membrane, enclosing a hyaline substance called the *nuclear-fluid*, or *nucleoplasm* (Fig. 22, *A*, *a*, *B*, *a*). In this nuclear fluid are found: the *nucleolus*, a spherical corpuscle made up of *pyrinin* to which the *chromatin*, a characteristic substance of the nucleus, frequently attaches itself; the *chromatic network*, the thread of which is made up of *linin*, a very slightly chromophilic substance, enclosing some grains, the *grains of chromatin*, which possess a special affinity for basic stains. The chromatin or *nuclein* is the most important substance of the nucleus.

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*By A. Guilliermond.

Centriole.—In intimate contact with the exterior of the nucleus and sometimes inside is usually found a small body called the *centrosome*, or, if the dense chromatin alone is considered, the *centriole* (Fig. 21, *B*, *a*). It is a small chromophilic grain which is often surrounded by a clear zone of protoplasm called *archoplasm*.

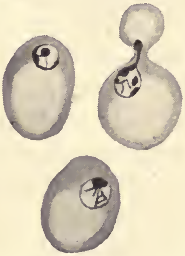

FIG. 3.

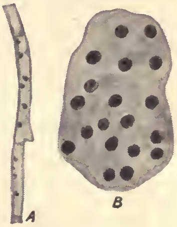

FIG. 4.

FIG. 3.—Cells of *Saccharomyces cerevisiæ.*

FIG. 4.—Cells made up of several energids. *A*, A portion of the mycelium of a mold, *Aspergillus ochraceus.* (*After Dangeard.*) *B*, Cell of an amœba, *Pelomyxa palustris.* (*After Doflein*).

Value of the Nucleus.—The nucleus is an organ indispensable to cellular life. It directs for the most part the physiological functions of the cell. It plays an active part in nutrition as is indicated by the fact that the greater part of the products of nutrition or of reserve spreads itself around the nuclear membrane. Finally, it assumes an important rôle in cellular division and in sexual phenomena.

The experiments of Balbiani which have been repeated by other authors show that the cell cannot function without its nucleus. By cutting an infusorial cell in two portions, one of which contains the nucleus and the other only its cytoplasm, Balbiani found that the nucleated part was able to resist the wound which it had received and regenerate the cytoplasm which was lacking; whereas the enucleated portion soon perished.

It does not seem probable, therefore, that cells can exist without their nuclei. Nevertheless, to the present time it has not been possible to find conclusive proof of the presence of a true nucleus in bacteria. The presence in their cells, however, of a great number of small chromatin grains like the chromatin material of nuclei, and their evolution during the formation of spores, force the observer to admit that these represent grains of nuclear substance, and that bacteria have a kind of *diffuse nucleus*, which is scattered in the form of small grains (Fig. 5) in the cytoplasm of the cell.

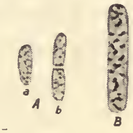

FIG. 5.—Diffuse nuclei of bacteria. *A*, *B. mycoides*. (*After Guilliermond.*) *B*, *Thiothrix tenuis*. (*After Swellengrebel.*)

Forms of Nuclei in Microörganisms.—The nucleus of primitive microörganisms is far simpler than in the higher forms, where it becomes fairly complex. Consequently in the *Cyanophyceæ* or blue-green algæ, the lowest of all algæ, the nucleus is in a very primitive state. It is large, not separated from the cytoplasm by a membrane, and is made up simply of a nuclear fluid and a chromatic network. The cytoplasm is confined to a thin cortical layer and the nucleus nearly fills the cell (Fig. 6).

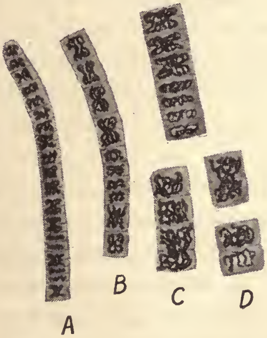

FIG. 6.—Nuclei of *Cyanophyceæ*. *A*, Thread of *Rivularia bullata* with nuclei in process of division. *B,–D*, Fragments of threads of *Calothrix pulvinata* showing nuclear division.

In other microörganisms the nucleus is much more complex. Yet frequently this nucleus is found in a primitive state quite different from typical nuclei of higher organisms. In some amœbæ, the nucleus is formed simply of a poorly defined membrane filled with nuclear fluid, and a large body of chromatin resembling a nucleolus called the *karyosome* or *centriole-nucleolus* (Fig. 22), because it acts both as a centriole and as a nucleolus. In the center of the karyosome is frequently seen a more intensely chromophilic corpuscle corresponding to the centriole (Fig. 21, *B*, *a*).

Many protozoa and some algæ have a centriole-nucleolus, but it is wholly enclosed in the nuclear fluid. The chromatin appears as little grains or as a network (Fig. 21, *A*, *a*).

In the higher microörganisms (protozoa and fungi) the nucleus begins to take the form of typical nuclei. The centriole detaches itself from the karyosome which becomes a true nucleolus, and may remain either wholly intranuclear (Fig. 20, *A*, *a*, 22, *A*, *a*), or become entirely extranuclear (Fig. 20, *B*, *a*, 22, *B*, *a*).

Theory of Binuclearity of Cells and Chromidia.—In the infusoria, the nuclear structure divides into two nuclei (Fig. 8); a large one, the *macronucleus* or *vegetative nucleus*, which functions during the vegetative life of the cell, and a small one lodged in a hollow of the macronucleus, the *reproductive nucleus* or *micronucleus*. At fertilization, the macronucleus is disorganized and its place taken by the micronucleus which reproduces by division both a micronucleus and a macronucleus. Certain flagellates have likewise two nuclei, a large vegetative and reproductive nucleus, and a small *micro-* or *kinetonucleus* which controls the formation of the flagellum.

Starting from these facts, a few investigators have tried to demonstrate that all cells have two nuclei. Recent evidence reveals that there are in the cytoplasm of most protozoa small chromophilic granules, like the chromatin material, which are supposed to emigrate from the nucleus during certain phases of development, and which are likened to the nuclear substance (Fig. 7). These granules are called *chromidia*, and all the granules scattered in the cytoplasm are designated as the *chromidial structure* or *chromidium*. Chromidia have been found in the cells of higher organisms. There is a theory that this chromidial system represents a second nucleus, the vegetative nucleus, scattered in the cytoplasm, and that the entire cell is provided with two nuclei, one of which has passed unseen up to this time because of its diffuse form. This theory is much doubted to-day, and it seems probable that the chromidium is simply a reserve material for the cell, or corresponds to formations which will be described later as *mitochondria*.

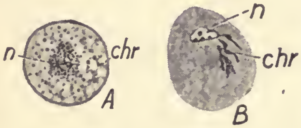

Fig. 7.—Chromidia in protozoa. *A*, The cycle of the microgamete of *Coccidium schubergi*. (*After Schaudinn.*) *B*, *Entamœba histolytica*. (*After Hartmann.*) *n*, Nucleus, *chr*. chromidia.

CYTOPLASM.—*Appearance and Properties of Cytoplasm.*—Cytoplasm may be defined for our purposes as a semi-fluid substance, granular in appearance, and reacting with an acid stain. It has three essential physiological properties, nutrition, motility, and sensibility. Cytoplasm appears to be composed largely of protein substances and of diverse lipoid substances in a state of colloidal solution. It varies widely according to circumstances, consequently it may be useless to search for any definite structure. In many microörganisms, as for example the protozoa, there is on the periphery of the cell a hyalin zone which is called the *ectoplasm* to distinguish it from the rest of the cytoplasm, the *endoplasm* (Fig. 17).

Chondriosomes.—Recent research has demonstrated special functioning bodies in the cytoplasm, the *mitochondria*, which seem to be the constructive elements of cytoplasm. They are a part of its structure, and are supposed to play an important physiological rôle in the cell. These structures, visible in the living organism, but stained only by a special process, are sometimes in the form of small isolated granules (*granular mitochondria*, Fig. 8, *B*), or of small threads (*thread-mitochondria*) or sometimes of rods much like certain bacilli (*rod-mitochondria*, Fig. 8, *A*). These forms frequently change from one to the other. The granular mitochondrium is able to elongate itself into a rod which is itself capable of dividing up into thread-mitochondria. All the mitochondria of one cell are called the *chondrium.* These structures seem to be made up of lipoidal substance and phosphates of albumin.

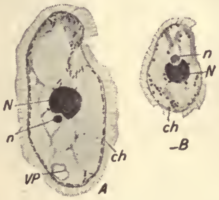

FIG. 8.—*Glaucoma piriformis*, infusorian with (*N*) macronucleus, (*n*) micronucleus, (*ch*) mitochondria, (*vp*) pulsating vacuole. (*After Fauré-Frémiet.*)

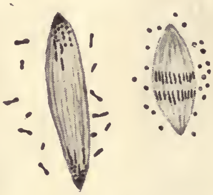

FIG. 9.—Division of micronucleus and of the chondriosomes in *Carchesium polypinum*, infusorian. (*After Fauré-Frémiet.*)

The mitochondria cannot generate themselves directly from the cytoplasm, but are formed always from preëxisting mitochondria by division. They apparently transmit themselves, after having divided, from the egg to the adult individual, and from the adult individual to the egg (Fig. 9).

Physiologically, mitochondria are organs of elaboration. In them, through some unknown physico-chemical phenomena, most of the products of cell activity may be formed. The product, whatever may be its specific nature, has its origin in a granular mitochondrium or in a rod-mitochondrium. Each product is surrounded by a mitochondrial exterior surface inside of which it develops slowly; the exterior surface remains until the product has reached its state of maturity.

It has been known for some time that there exist in higher plants corpuscular elements called *plastids* or *leucoplastids*, which also possess a synthetic function. Some, the *chloroplastids*, make the chlorophyl which, by using rays of light as energy, forms starch; others, the *amyloplastids*, confine themselves to forming starch from the excess sugars found in the cells; still others, the *chromoplastids*, constitute the pigment bodies of plants (xanthophyl, carotins). It has been recently shown that plastids are nothing but mitochondria which have undergone greater differentiation and specialization than those which, at the expense of ordinary mitochrondria derived from the egg, have increased in size (Figs. 10, 11).

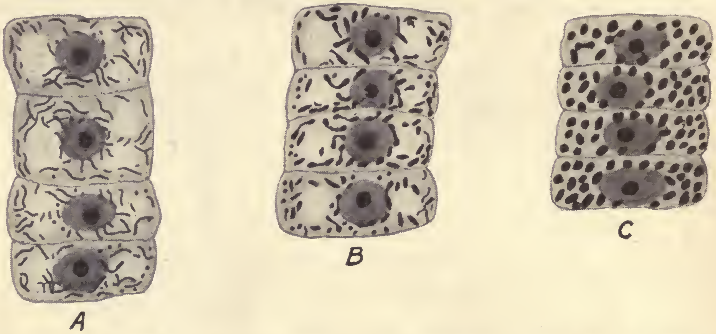

FIG. 10.—Formation of chloroplasts in the young leaf of barley. *A*, Very young cells in which appear rod-mitochondria. *B*, Older cells in which the rod-mitochondria are transforming themselves into chloroplasts. *C*, Cells in which the chloroplasts are definitely constituted.

Mitochondria have been found in most protozoa and fungi. In the latter they take part in the formation of reserve products, especially the *metachromatic corpuscles* of which more will be said later.

Mitochondria are most highly developed in algæ where they give origin to chloroplastids as in higher plants. On the other hand, in the lower forms, no mitochrondria seem to exist, but the chloroplastids take on certain special characteristics. Instead of small scattered corpuscles is found one, or occasionally several, large chloroplastids filling most of the cell. They are in various shapes—ribbons, spirals, nets, etoilated bodies (Fig. 12), etc.—but all appear to be made up of a mitochondrial substance. Their physiological rôle is much more general than in the chloroplastids of higher plants. They produce not only the chlorophyl, but other pigment bodies, the starch or paramylum, metachromatic corpuscles, and globules of fat. Consequently the complex chloroplastids of the algæ with their general function have been considered as a special form of chondrium which, instead of being scattered in the cytoplasm as a number of small structures, finds itself gathered in very compact masses.

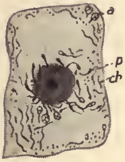

FIG. 11.

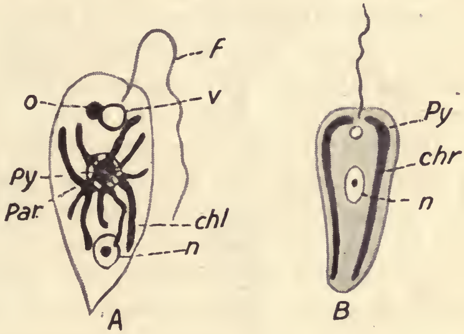

FIG. 12.

FIG. 11.—A cell from the root of a bean in which the rod-mitochondria (*ch*) form in the course of their development amyloplasts from which (*p*) spring grains of starch (*a*).

FIG. 12.—*A*, *Euglena viridis* with its star-like chloroplasts (*chl.*) at the center of the organism, the pyrénoid body (*Py*) surrounded by grains of paramylum (*Par*). eye-spot (*o*), contractile vacuole (*v*), flagellum (*f*), nucleus (*n*). (*After Dangeard.*) *B*, *Microglena punctifera*, with two elongated chromatophores arranged longitudinally. (*After Stein.*)

The *Cyanophyceæ* are the only microörganisms in which the chondrium has not been found. In the *Cyanophyceæ* the chlorophyl and the blue pigment (phycocyanin) associated with it are diffused throughout the cytoplasmic area surrounding the nucleus. The very primitive structure of the algæ explains to some extent this absence of an important structure of the cell.

Vacuoles.—There is always in the cytoplasm one (or several) rather bulky vesicle filled supposedly with an aqueous solution of mineral salts called a *vacuole.* Vacuoles play an important part in the absorption of liquids by the cell. Owing to the mineral salts dissolved in the vacuole-fluid, the concentration of which is ordinarily higher than that of the surrounding medium, the vacuoles become the center of osmotic forces which consequently cause a part of the ambient liquid to penetrate the cell and determine its turgescence.

Very curious vacuoles are found in many protozoa, namely, the *pulsating vacuoles* (Figs. 8, 12). They are small vacuoles which expand and contract rhythmically, and which are considered as excretory and respiratory organs. The water that has entered the cell gathers in this vacuole and is expelled as it contracts. Probably in crossing the body this water yields its oxygen to the cytoplasm in order to charge itself with carbonic acid and the products of metabolism.

Reserve Products.—The cytoplasm encloses some structures differentiable by means of certain stains or chemical reagents as granulations, but which are not constituent elements of cytoplasm; they come from a secretion of the cytoplasm, and only under certain conditions. These grains may be found either in the cytoplasmic substance itself, or in the vacuoles included in the cytoplasm. Most of these granules are reserve products which appear when nutrition is deficient. Among the reserve products most common in microörganisms are the granules called *metachromatic corpuscles* (Fig. 13, *A*). These bodies, which are the object of a special study in connection with molds and yeasts, are made up of a substance the nature of which is still unknown, and are found in nearly all fungi, in most algæ and bacteria, and in many protozoa.

Glycogen and paraglycogen are equally well distributed in microörganisms (fungi, protozoa). Among algæ, glycogen is found only in the *Cyanophyceæ,* but it is elsewhere replaced by starch or paramylum (Fig. 11), common products of chlorophyllic assimilation.

There are also the protein substances, such as crystalloids of *mucorin* scattered in the *Mucorinæ,* or the globules of fat common in all cells (Fig. 13, *B*).

Most of these substances seem to result from the activity of the chondrium structure. Recent investigation shows that the metachromatic corpuscles have their rise among the mitochondria. It has long been known, on the other hand, that the starch and paramylum are always formed in the chloroplastids.

MEMBRANE.—The cell is usually enveloped in a more or less heavy membrane, secreted by the cytoplasm, which acts as a protective organ for the cell.

The presence of the membrane is not, however, indispensable; many protozoa do not have it, and are consequently naked cells. Motility in many microörganisms is closely associated with the membrane, for the movement of cytoplasm and the flexibility of the membrane are essential factors. Cells as a rule have a membrane of different degrees of thickness and composition. It may be albuminoid or chitinous (*Infusoria*), or it may be made up of carbohydrates, as cellulose, pectose, and callose (algæ, fungi). Bacteria always have a membrane, but its nature has not yet been definitely determined. Often the cell membrane is able to thicken noticeably, and thus protect the cell from influences of environment; the cell may then be regarded as transformed into a cyst which passes into a state of sluggish existence. Encystment is frequent with protozoa, and is produced when the environment becomes unfavorable (Fig. 14, *A*).

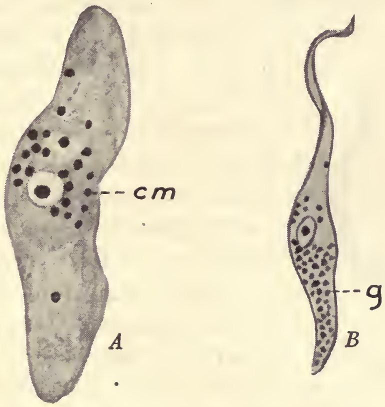

FIG. 13.—*A*, Metachromatic corpuscles (*cm*) in *Sarcosporidia*, *Sarcocystis tenella*. (*After Erdmann.*) *B*, Fat globules (*g*) in *Trypanosoma rotatorium*. (*After Doflein.*)

The external layer of the membrane frequently undergoes modifications, transforming itself into a mucilaginous or gelatinous substance as we see in many *Cyanophyceæ*, in bacteria surrounded by capsules, and in zoöglea. The membrane then becomes extremely thick (Fig. 14,*B*).

LOCOMOTIVE STRUCTURE.—Most algæ and fungi cannot move. Many bacteria and all protozoa have more or less perfected locomotive structure.

The *Cyanophyceæ* and many bacteria, although without locomotive organs, present nevertheless oscillatory movements which seem due to a general movement of the cytoplasm translated exteriorly because of the flexibility of their membrane. With these exceptions, movement is effected by means of a locomotive structure.

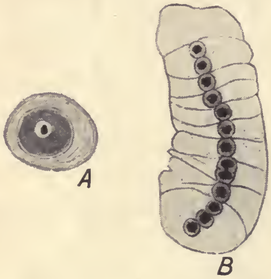

FIG. 14.—*A*, Cyst of *Amœba limax.* (*After Dangeard.*) *B*, Thread of nostoc surrounded by a thick mucilaginous case.

This structure is found in its simplest form in the *pseudopodia* of the amœba. The naked cell of the amœba pushes out pseudopods, simple expansions of the ectoplasm arising at any part of the body, which take various shapes, and reënter the body without leaving the least trace of their existence. It is a result of motility of the cytoplasm, one of its essential properties, shown here exteriorly because of the absence of a cellular membrane.

The locomotive structure is more complex in other protozoa; the pseudopod is replaced by contractile appendages—*flagella*, or *vibratile cilia.*

The flagellum is a contractile appendage of definite shape and position which draws the body after it by means of waving movements. It is found on bacteria and flagellates.

The organ of locomotion of bacteria is still little known (Fig. 15). It consists of a certain number of contractile appendages placed at one end of the cell, or at both, or sometimes distributed over the whole body. These appendages, which may be called vibrating appendages, have the characteristics of flagella. Their existence, for a long time doubted, is now well established.

The locomotive structure of the *Flagellata* is much better known. It is characterized by one or more flagella inserted in the anterior extremity of the cell. In case of more, one frequently folds back toward the posterior end. In the lateral region of the cell it unites with a contractile membrane, the *undulating membrane*, running in spiral form along the length of the body, of which it is the free end. Flagella are made up of one or more elastic fibers, surrounded by a thin cytoplasmic sheath.

The vibrating cilia are also contractile appendages, differing from the flagella only in their smaller size. They cover the whole body of the cell, as in the case of infusoria, enabling them to move about very easily in liquids. This interpretation is not concurred in by all investigators.

Certain facts lead us to believe that flagella are only transformed pseudopods in which the cytoplasmic structure has changed and at the same time the kind of movement. Thread-like pseudopods are found with a rapid rhythmic movement which may serve as intermediate forms. Be that as it may, the method of forming these organs is of special interest. Apparently they are formed under the influence and at the expense of the centriole.

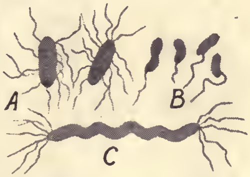

FIG. 15.—Organs of locomotion in bacteria. *A*, *B. subtilis*. (*After Fischer*) *B*, *Microspira comma*. (*After Fischer and Migula*.) *C*, *Spirillum rubrum*.

In the *Flagellata* the flagellum is always inserted in the centriole or in a similar organ which appears to issue from the centriole. It is not rare to find in cellular division some cells in which the nucleus is dividing with a centriole at each of its poles. Each serves as a point of insertion for a flagellum (Fig. 16, *A*, *D*, *E*).

According to recent works, the flagellum is formed in general in one of two somewhat different methods.

In the first case, the centriole divides itself by an elongation, followed by a contraction into two centrioles which remain united to each other by means of a fine thread, the *centrodesmose*. The centrodesmose then elongates and is transformed into a flagellum.

In the second case, the centriole divides itself a first time just as in the preceding case, but the centriole farthest from the nucleus immediately undergoes a second division, thus making three centrioles. The one nearest the nucleus remains a centriole during nuclear division. The centriole situated somewhat farther from the nucleus becomes the point of insertion for the flagellum, and is called the *blepharoplast* or *basal* *grain.* The centriole is united to the blepharoplast by a *centrodesmose*, the *rhizoplast*, which is often absorbed. Finally, the last centriole situated beyond the blepharoplast about equally distant, also unites with this cell-organ by a centrodesmose and, by approaching the extremity of the cell, causes the elongation of the centrodesmose which transforms itself into a flagellum.

In the infusoria the vibratile cilia insert themselves in the ectoplasm and pass through the cuticle to reach the exterior. At the point of insertion of each of these cilia is a small chromatic corpuscle or basal grain, a *trichocyst*, also supposed to arise from a repeated division of the centriole (Fig. 17).

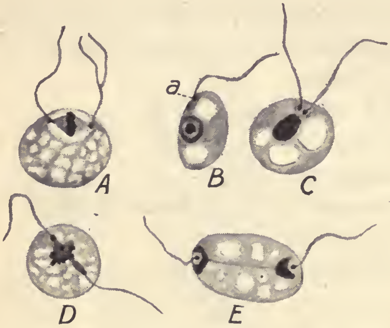

FIG. 16.

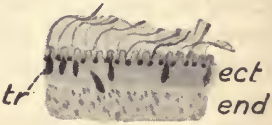

FIG. 17.

FIG. 16.—*A*, *Spongomonas uvella.* The nucleus is undergoing mitotic division. Two centrioles, each at the base of a flagellum, are located at the two extremes of the spindle. (*After Hartmann and Chagas.*)

B, *Monas termo.* The cell lies in repose; a centriole (*a*) lies at the base of the flagellum; in (*C*) there are two centrioles, in (*D*) the two centrioles occupy the two poles of the nucleus during the process of mitosis; in (*E*) exists the final nuclear division. (*After Martin.*)

FIG. 17.—Fragments of the peripheral portion of *Prorodon teres* (infusorian) with vibratile cilia and their basal corpuscles. (*ect*) Ectoplasm; (*end*) endoplasm; (*tr*) trichocysts. (*After Maier and Gurwitch.*)

The centriole which, as we shall see later, seems to be a motor organ associated with the internal cytoplasmic movements during cellular division, appears also to be connected with the external movement of the cell.

REPRODUCTION OF THE CELL

VARIOUS PROCESSES OF REPRODUCTION.—Reproduction of microbes is affected by various processes; the cell may reproduce itself by transverse or longitudinal fission, binary division, schizogony (bacteria, flagellata, molds, Figs. 6, *A;* 18; 20, *A*). This is by far the most frequent. It sometimes, however, divides itself by *budding, gemmulation* (Yeast, Fig. 3); that is, by the formation of a small protuberance which separates itself from the mother cell as a small daughter cell which, once free, grows slowly to maturity.

Finally, a last process and a very frequent one is the formation of internal spores, or *sporogony* (Fig. 19). The nucleus undergoes a certain number of divisions, and the cytoplasm divides itself inside the cell in as many small cells as there are nuclei. These cells become spores and are set free by a rupture in the wall of the mother cell. Sometimes all the cytoplasm of the mother cell divides into spores, and sometimes only a part of the cytoplasm is used, the rest *epiplasm* serving as nourishment to the spores during their growth.

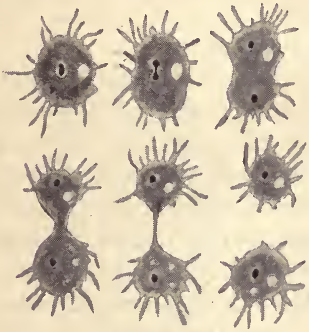

FIG. 18.—Schizogony in *Amœba polypodia* with amitotic division of the nucleus. (*After Schulze and Lange.*)

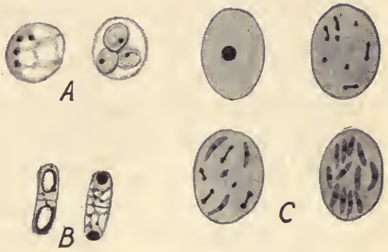

FIG. 19.—Sporogony. *A*, Formation of spores in *Saccharomyces cerevisiæ*. *B*, Formation of spores in *B. mycoides*. (*After Guilliermond.*) *C*. Formation of spores in *Leucocytozoon lovati*. (*After Fantham.*)

Whatever the means by which the cell reproduces itself, cytoplasmic changes and nuclear changes take place at the same time. The most important of the cytoplasmic changes is the distribution of the chondrium structure between two daughter cells, often preceding the division of this cytoplasmic structure (Fig. 9).

The nuclear phenomena are much more important, and better known. The nucleus divides in order to furnish each daughter cell with a nucleus containing the same amount of chromatin.

Nuclear Division.—Nuclear division may occur in one of two ways, one very complex, (1) the *indirect mode, karyokinesis* or *mitosis;* the other very simple, (2) the *direct mode*, or *amitosis.*

Indirect Division, Karyokinesis, or Mitosis.—We shall begin with the indirect mode which is by far the more common, using as an example a *Heliozoön*, the *Acanthocystis aculeata* (Fig. 20, *A*). The nucleus of this protozoon at rest contains a large karyosome of a spongy structure, and a chromatic network. Outside the karyosome in the nuclear vesicle is a centriole surrounded by a hyaline zone, the archoplasm (Fig. 20, *A*, *a*).

Mitosis may be divided into four steps or phases.

The *first phase* or *prophase* begins by the emigration of the centriole from the nucleus outside of which it surrounds itself by cytoplasmic irradiations, making a star-like body, called the *aster* (Fig. 20, *A*, *b*). Following this, the karyosome dissolves in the nucleoplasm, supposedly conveying material to the chromatic network which enriches itself noticeably in chromatin. The chromatic network then relaxes, thickens and transforms itself into a more or less spiral cluster, the *spireme* (Fig. 20, *A*, *c*). At the same time the centriole divides into two centrioles, each surrounded by an aster (Fig. 20, *A*, *c*). Soon these centrioles place themselves at the two opposite poles of the nucleus (Fig. 20, *A*, *d*), while the spireme breaks itself up into a definite number of chromatic sections, the *chromosomes.* While this is taking place, the nuclear membrane dissolves itself into a series of cytoplasmic fibrils, the *achromatic spindle*, resistant to nuclear stains. They appear in the middle of the nucleus and converge at each end to the centrioles (Fig. 20, *A*, *d*, *c*). The chromosomes group themselves in the center of the spindle as the *equatorial plate* (Fig. 20, *A*, *e*), the formation of which completes the prophase. Each of the chromosomes is attached to one of the fibrils which make up the achromatic spindle.

The *second phase* or *metaphase* consists of the longitudinal division of the chromosomes each of which divides itself into two equal chromosomes.

In the *third phase* or *anaphase* the chromosomes equally divided move to the two poles where they make two polar plates. The centrioles located here seem to have some attraction for the chromosomes.

Finally comes the *telophase* or phase of reconstitution of the two nuclei which terminates the process. In this phase, the chromosomes

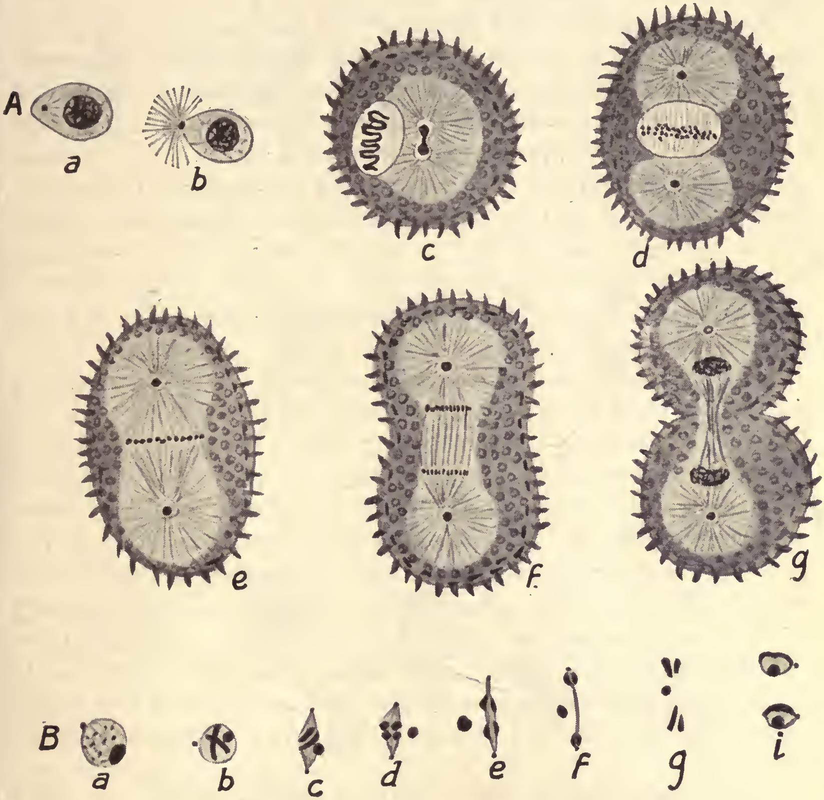

FIG. 20.—Karyokinesis (metamitosis). *A, Acanthocystis aculeata;* (*a*) nucleus in state of repose with an intranuclear centriole; (*b*) (prophase) the centriole moves to the periphery and out of the nucleus and forms an aster (*After Hertwig*); (*c*) the division of the centriole and spireme; (*d*) the formation of the equatorial plates and the achromatic spindle; (*e*) equatorial plates; (*f*) anaphase; (*g*) telophase. (After Schaudinn.) *B*, In *Coleosporium senecionis* (Uredineæ). (*a*) Nucleus at rest with its centriole extranuclear; (*b*) formation of chromosomes; (*c*) equatorial plate; (*d*) metaphase; (*e*) anaphase; (*f*) (*g*) (*i*) telophase. (*After Madame Moreau.*)

form a spiral chromatic cluster making a spireme at each of the poles (dispireme stage, Fig. 20, *A*, *g*); each of the spiremes is then surrounded by a nuclear membrane in which is included the centriole. Thus the two nuclei are formed in which a nucleolus soon appears. Meanwhile the cell has elongated, become constricted in the center, and finally broken into two cells (Fig. 20, *B*, *f*, *g*, *i*). The achromatic spindle completely disappears.

This method of division represents the typical method of karyokinesis, that which is observed in higher organisms with the single difference that the centriole is intranuclear, whereas in the cells of higher organisms it is ordinarily outside the nucleus in contact with the nuclear membrane. An analogous mitosis is found in the *Uredineæ* (Fig. 20, *B*, *a-i*), except that the centriole is here found to be extranuclear (Fig. 20, *B*, *a*), the asters are lacking, and the nucleolus persists to the end of mitosis expelled in the cytoplasm. The physiological significance of the nucleolus in this case is not known. This method of division is seen in certain molds and higher protozoa, and is called *metamitosis* or *perfect mitosis.*

Summing up, mitosis is a process functioning to make an absolutely equal division of the chromatin between the two nuclei. This distribution is performed by the breaking up of a spireme into a definite number of chromosomes, a number varying according to the species but always constant for any single species, and then by a longitudinal division of the latter. The centrioles seem to play an important rôle in this phenomenon, in directing it, and in attracting the chromosomes once divided toward the poles of the cell where the nuclei are formed.

It is not necessary to conclude that the processes of mitosis are as complex as in other microörganisms. Relatively simple in the lower forms, mitosis becomes complicated as it climbs the ladder, gaining the characteristics of metamitosis only in the most advanced forms.

The simplest case is found in the *Cyanophyceæ* (Fig. 6). Here cellular division begins by the outline of the transverse partition which appears in the form of a peripheral ring. At the same time the chromatic network takes a definite arrangement; its filaments arrange themselves parallel to the longitudinal axis of the cell, thus giving this division the appearance of a mitotic division. The outline of the partition extends little by little toward the middle of the cell, leaving open only a small spherical space in its center to which the fibers of the network then contract, and the nucleus takes the form of

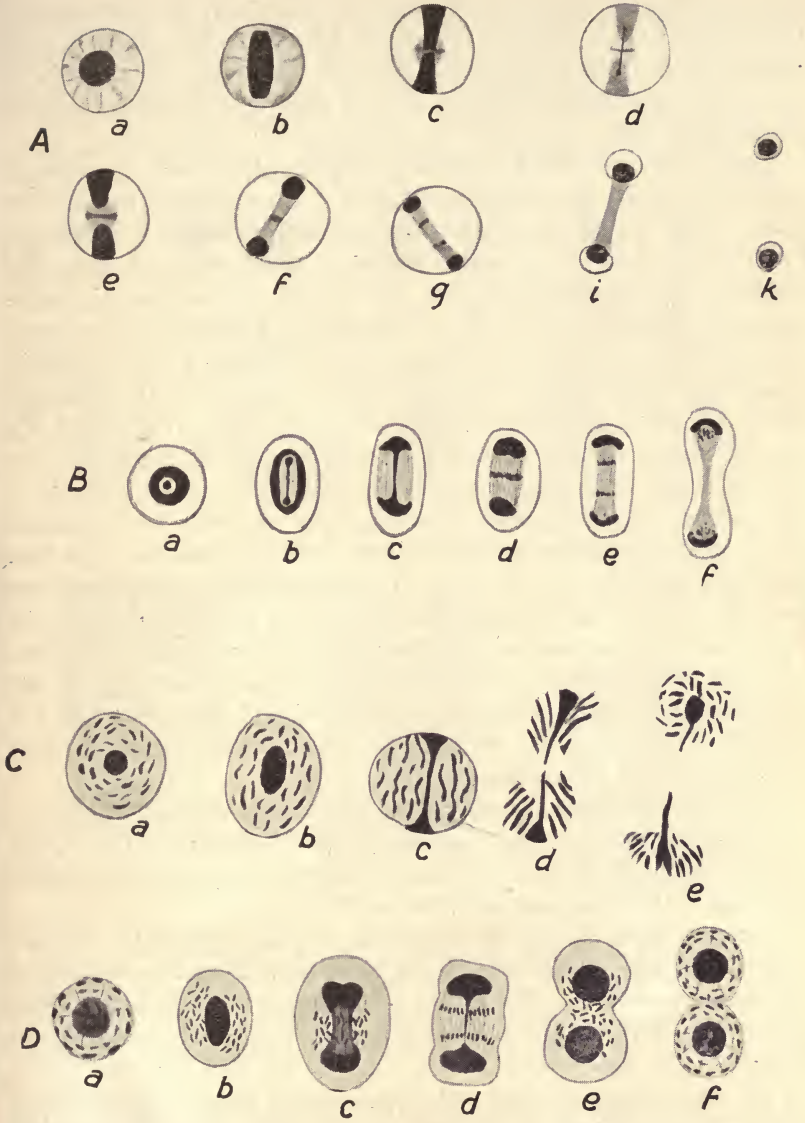

FIG. 21.—Protomitosis. *A*, In *Amœba mucicola.* (*a*) Nucleus at rest; (*b*) beginning of prophase; (*c*) division karyosome; (*d*) division of centriole; (*e*) (*f*) equatorial plate; (*g*) metaphase, (*i*) (*k*) telophase. (*After Chatton.*) *B*, In *Amœba froschi.* (*After Nägler.*) *C*, In *Euglena splendens.* (*After Dangeard.*) *D*, In *Amœba diplomitotica.* (*After Beaurepaire Arago.*)

a dumb-bell. Soon the partition stops completely, the filaments of the contracted part of the nucleus break up and the two daughter cells appear separated by a partition. The two nuclei whose filaments have been sectioned by the partition are not slow in recovering their integrity (Fig. 6, *b*).

We find in the *Amœba mucicola* (Fig. 21, *A*) a much more characteristic mitosis, though more primitive. The nucleus of this amœba when at rest is made up of a nuclear fluid surrounded by a membrane in which are a large karyosome and some small grains of chromatin localized on the periphery (Fig. 21, *A*). In the center of the karyosome is a small chromophilic centriole. The prophase begins by the elongation of the karyosome to a rod-shaped body (Fig. 21, *A*, *b*) which then transforms itself into a dumb-bell (Fig. 21, *A*, *c*). The centriole also elongates and becomes constricted in the center (Fig. 21, *A*, *d*). At the same time an achromatic spindle appears all about the constricted region of the karyosome in the middle of which the grains of chromatin arrange themselves peripherally to form an equatorial plate, but there is no differentiation of this chromatin into two chromosomes (Fig. 21, *A*, *c*, *d*). In the metaphase the karyosome and the centriole divide into two polar masses (Fig. 21, *A*, *e*, *f*), the equatorial plate separates into two plates which, in the anaphase, emigrate to the poles (Fig. 21, *A*, *g*) drawn by the centrioles. In the telophase the spindle elongates, disappears, and the two nuclei are formed at the poles (Fig. 21, *A*, *i*, *k*). The nuclear membrane exists during the entire phenomenon.

In other microörganisms (*Amœbæ*, *Flagellata*, *Euglenæ*) is found a similar mitosis except that the chromatin distributed in the resting nucleus as a network or as rod-shaped bodies forms an equatorial plate made up of true chromosomes (Fig. 21, *B*, *C*).

Another form of mitosis, *promitosis*, is characterized by the fact that the centriole is included in the karyosome, by the persistence of the nuclear membrane, and by the simultaneous division in the metaphase of the karyosome and of the chromatin gathered in an equatorial plate.

Between promitosis and metamitosis are a series of intermediate forms. In the *Pelomyxa palustris*, for example, the centriole while remaining intranuclear is able to separate itself from the karyosome (Fig. 22, *A*, *a*). The prophase here begins with the usual division of the centriole (Fig. 22, *A*, *b*, *c*), and the two resulting centriole-threads pass to the extremities of the achromatic spindle, while the karyosome coöperates in the formation of the chromosomes (Fig. 22, *A*, *d*, *e*).

In other cases (various fungi, *Gregarinæ*, etc.), the centriole becomes extranuclear, and the karyosome acts as a true nucleolus (Fig. 22). Sometimes it dissolves at the beginning of mitosis, seeming to aid the development of the chromatin of the spireme, and sometimes it persists during the entire process and is expelled in the cytoplasm at the end of the phenomenon without any known function. The

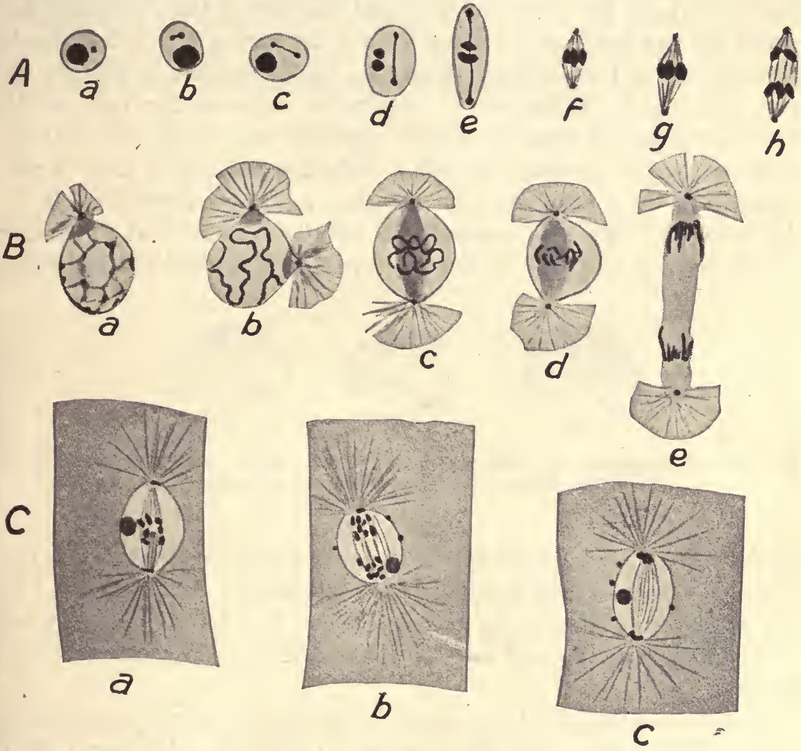

FIG. 22.—Mesomitosis. *A*, In *Pelomyxa palustris*. (*a*) Nucleus at rest; (*b*)—(*e*) division of centriole; (*f*) (*g*) equatorial plate; (*h*) anaphase. (*After Bott.*) *B*, In *Urospora lagidis* (*Gregarina*). (*a*) Nucleus with extranuclear centriole and aster; (*b*) the centriole is divided and the spireme is formed; (*c*) spireme; (*d*) equatorial plate; (*e*) anaphase. (*After Brasil.*) *C*, In the ascus of *Galactima succosa* (*Ascomycete*). (*a*) Equatorial plate; (*b*) anaphase; (*c*) telophase.

name of *mesomitosis* has been given to all the mitoses which distinguish themselves from promitosis by the persistence of a nuclear membrane throughout the phenomenon.

Direct Division or Amitosis.—This consists simply of an elongation of the nucleus followed by a median constriction, then by a rupture of this constricted part without an equal division of the chromatin between the two nuclei which often are not the same size. It is a simple breaking up of the nucleus. Amitosis, then, does not necessarily insure the equal distribution of chromatin between the two nuclei. This rare process is found in higher organisms only in old cells that are degenerating, or in diseased cells. Although for a long time it was thought to be a primitive phenomenon, it is now considered to be degenerative. We see, however, in certain *Amœbæ* and *Mycetozoa* the karyosome enclosing all the chromatin divides itself into two equal bodies, showing the characteristics of a very primitive mitosis (Fig. 18). Amitosis seems to exist normally in yeasts and in certain molds. In the yeasts, for example, the nucleus divides by amitosis in the course of budding (Fig. 3), and mitosis is found only in the course of sporulation.

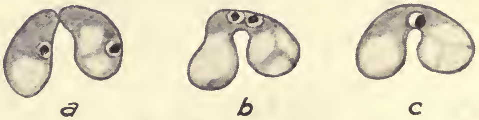

FIG. 23.—Conjugation in *Schizosaccharomyces octosporus*. (*a*) Two gametes in the process of fusion; (*b*) (*c*) nuclear fusion.

SEXUAL CHANGES.—In most microörganisms at certain times during their existence occur sexual changes, or fertilization, which seem to give them a new strength. It is followed by a period of very active reproduction, whence the name of sexual reproduction given to these changes. This consists essentially in the fusion of two equal *isogamous* (*isogamy*) or unequal, *anisogamous* (*heterogamy*) cells or *gametes*. In the latter case, the male is small and active, and the female large and passive. The fusion between the two cytoplasms and the two nuclei takes place at the same time (Fig. 23).

If nuclear fusion were not compensated by an elimination of chromatin, the nucleus would increase in this substance at each fertilization. But this change is succeeded immediately in protozoa by a common process called *chromatic reduction.* The chromosomes in the course of the divisions which precede the formation of the gametes reduce themselves to half by a complex process which it would be superfluous to describe here. The same chromatic reduction takes place in the fungi and algæ, but this does not always precede fertilization. It may follow it immediately as in the yeasts where it seems to produce itself during the nuclear divisions in the ascus. It may also occur during other stages of development.

CHAPTER II*

MOLDS

Fungi in General

Certain fungi commonly designated molds are constantly met in microbiological studies. They are found as contaminating colonies in microbial cultures. They are agents together with other microörganisms in the processes of fermentation and decay in the soil, and in the spoilage of food-stuffs. Certain of these forms approach the structure and habits of other microörganisms very closely. Members of these border groups have been sometimes described as bacteria, sometimes as fungi; among them the *Actinomycetes* have been principally studied by bacteriologists, but recently Drechsler has succeeded in describing their fruiting forms in terms which leave no doubt that they are a fungous group. In describing microörganisms, cultural reactions have been largely used in characterizing the bacteria and related organisms; morphology has been the basis of nearly all descriptions of the mycelial fungi.

With some exceptions, there is, among the cells of the true fungi, a differentiation of function into vegetative or assimilative cells and reproductive cells. The fungous body is usually composed of threads (technically called *hyphæ*, singular, *hypha*). These *hyphæ* usually branch in more or less complex manner forming networks or webs, collectively called *mycelium*. Hyphæ may be one-celled or composed of many cells placed end to end as shown by the cross walls, called *septa*, seen in them. These threads grow either by the formation of new cells at the growing tips (called *apical growth*) or by the division of cells in the hypha (*intercalary growth*). The fungous cells rarely divide in three planes to produce solid masses of cells. Both vegetative and reproductive masses are formed in great variety from such hyphæ. Often the thread-like character is almost or quite obliterated in the ripe masses, which may be fleshy, woody, carbonaceous, leathery and even horn-like in texture, as seen especially in the mushrooms, bracket-fungi, etc., but even in such cases the early stages show the structures to originate from masses of fungous threads.

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* Prepared by Charles Thom. A. Guilliermond has furnished the sections on "Cytology of Molds."

The formation of differentiated reproductive cells is, in general, characteristic of the fungi. The method of reproduction presents great variety. In the simplest forms, the reproductive cells are scarcely, if at all, distinguishable from the vegetative cells. In some species whole hyphæ break up so that each cell forms the starting-point of a new colony. Other forms develop special branches bearing reproductive cells. From these it is but a step to the production of fruiting branches, characteristic in form, called *conidiophores*, bearing cells markedly specialized as reproductive by form and frequently also by color, called *conidia*. These conidia are entirely asexual in origin and capable of growing directly into new colonies, although in many cases they are provided with resistant walls which enable them to live for long periods if conditions are unfavorable to growth at once. In other species, specialized resting cells with resistant walls are formed to enable the plant to survive unfavorable conditions. These are called *chlamydospores* or sometimes *cysts*. The name *gemmæ* is sometimes applied to similar structures, preferably to such as grow at once. The same end is reached in still other groups by the formation of *sclerotia* which are hard masses or balls of thick-walled cells filled with concentrated food materials. These sclerotia are frequently distinctive of the species producing them by size and appearance. They vary from microscopic in size to masses weighing many pounds and may perhaps in cases be aborted fruits. They sometimes resemble such sexual fruiting bodies. Resting structures of either type, especially when large, commonly produce typical spore-bearing structures at once after germinating. Sexuality among the fungi has been difficult to demonstrate in some groups with complex fruit bodies. It is certainly suppressed, if not entirely wanting, in whole series of cosmopolitan forms and present only in rare species in other groups.

The systems of classification used are largely based upon the types of sexual fruit bodies produced. Where such fruit bodies are not known, the method of formation of the asexual spores furnishes the most satisfactory basis for grouping. In classifying fungi, certain types of spore formation are found to be characteristic of particular groups.

Since within these groups various accessory types of fruiting occur, so that some species show three or even more forms of spores, that type of spore formation which is regarded as characteristic of the group is known as the perfect stage. If sexual fruits are found, these constitute the perfect stage of the group; if no such fruit is found, the most characteristic asexual form is used.

Between the typical forms are many gradations resulting in many families whose relationship to one or the other group is difficult to determine. Probably the ancestral history (phylogeny) of the fungi, if known, would show several or many lines of descent rather than one. Many thousands of species of fungi have been described, principally in Latin, German, French and English. The literature is widely scattered in monographs, reports and journals published in various languages. For the purposes of the bacteriological worker, a few representative groups with some of the significant species will be considered here.

Bacteria.—In the scheme of plant grouping presented (page 13), which is only one of many attempts to show relationships, the bacteria are placed with a group of single-celled green or blue-green forms as Schizophyta or fission-plants because of reproduction only by the division of the cells. Recent work of Löhnis, Cort and others have opened possibilities of specialized spore-production in the bacteria. Such reproductive bodies, if proved present and fully described, would probably furnish a sound basis for a scheme of relationships of the bacteria. At present it is undecided whether they are specialized from higher forms by suppression of characters or represent a primitive morphology with highly specialized physiological relations.

Phycomycetes.—The *Phycomycetes* are called algal fungi because they resemble certain groups of green filamentous forms in many particulars. In this group two general types of sexual reproduction appear—zygospore formation and oöspore formation. The first, found in the *Zygomycetes* represented by the common mucors, consists of the fusion of terminal cells of branches of the mycelium similar in appearance but differentiated in sex. As a result of this fertilization large thick-walled resting cells are produced, called *zygospores*, from a Greek root meaning yoked (Fig. 33). In oöspore formation, found in the *Oömycetes*, the conjugating cells differ in appearance as well as in function. The *oöspore* or egg-cell is large and is rich in food materials; the *antheridium* is much smaller, penetrates and fertilizes the oöspore, which afterward develops into a thick-walled resting spore. The very destructive downy mildews belong to this group.

Ascomycetes.—In this great group sexuality was denied until recent years, but has been proved in cases enough to establish a presumption of more general occurrence. The characteristic structure of the group is the *ascus*, a sac containing, when ripe, typically eight spores, sometimes a less number by the failure of some to develop, sometimes a larger number, usually some multiple of eight. The ascus when sexuality is known is developed subsequent to fertilization, not directly from an egg cell. The group presents a great variety of fruiting masses produced in connection with the asci. The simplest forms are loose webs of hyphæ enmeshing a few asci; other forms show clubs, cups, flask forms, crusted areas, the type of mass in each case being characteristic of the family, genus and species represented. Only a few of many thousands of these forms are encountered in bacteriological work. One genus is, however, constantly found. The commonest species of *Aspergillus* produces bright yellow, globose fruiting bodies, called *perithecia* (singular, *perithecium*), filled with asci. These are borne upon the surface of the substratum and often give a yellow color to the colony by their abundance. Such perithecia consist of the ascogenous cells and the asci produced by them, about which a more or less completely closed sac or wall has been formed, by the development of the sterile cells adjacent to the fruiting ones.

Basidiomycetes.—In the *Basidiomycetes* there is still further reduction of the evidences of sexuality.

In some sections of this group an essential sexuality has been correlated with the fusion of nuclei at stages of life history characteristic for the particular sections of the group. Such fusion seems to underlie the development of the typical faint body, although it has only been demonstrated in a small number of species. The typical structure is the *basidium*, a spore-bearing cell characteristically producing four protuberances called *sterigmata* (singular, *sterigma*), each bearing a single spore. These basidia are grouped into many kinds of fruit bodies varying from occurrence here and there upon a loose web of hyphæ to dense columnar areas covering the gills of the mushrooms or lining the cavities of the puffballs. Very few of these species are encountered in bacteriological studies.

IMPERFECT FUNGI.—A very large number of species are known which have never been seen to produce sexual fruits or fruits characteristic of the great groups. These are brought together and described as form-genera by their method of asexual spore formation. From the lack of the organs used in classifying the other groups, these are called the *Imperfect Fungi* and their grouping regarded only as temporary, a convenience for the identification of materials. These include many forms of economic importance, and many of the species most frequently met in bacteriological work. Sometimes one or a few species of a large group produce the perfect form while very many species cannot be induced to do so. Some of these species undoubtedly represent stages of perfect fungi whose perfect forms simply are not recognized as connected with these; others as in the more common species of *Penicillium* reproduce for an indefinite number of generations by conidia. Such species do not appear to need the perfect form and hence apparently have, in some cases, lost the power to produce it. Genera consisting entirely of such species are very properly retained as form-genera in the *Imperfect* group.

As found in nature all these forms are parasitic, saprophytic, or capable of both modes of life. All depend more or less completely upon organic matter for nourishment. Great diversity exists, however, in their adaptation to environment. Many of them are not only parasitic but so closely adapted to parasitizing particular host-species as not to be found elsewhere. Others attack several or many species, usually related. Even among saprophytes many species are found only upon particular forms of decaying animal or vegetable matter. The great economic importance of these parasitic and closely adapted saprophytic species has been recognized by the development in recent years of the literature of plant pathology (phytopathology). These cannot be considered in this work.

CYTOLOGY OF MOLDS*

GENERAL STRUCTURE OF MOLDS.—Three kinds of cell-structure formation are found in molds:

1. Some, belonging to the *Phycomycetes*, show no cross-walls; they have a much branched, felted mycelium, but in the early stages there

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* Prepared by A. Guilliermond.

are no true transverse septa. Septa appear in many forms only when fruiting begins, but in the opinion of some they merely separate the living portions of the mycelium from those in which the cytoplasm is dead or degenerating. The cytoplasm in the unseptate mycelium forms one continuous mass; it contains a great many nuclei (Fig. 24, 1 and 2). Each nucleus with the cytoplasm surrounding it, according to Sachs, may be considered a physiological unit acting in a somewhat similar capacity as a cell, or may be designated as an *energid*. This view is not held by all observers, however. Considered thus, the mycelium represents the collection of a great many indistinct cells which are not separated by walls. The *Mucorineæ*, for example, belong to this structural type.

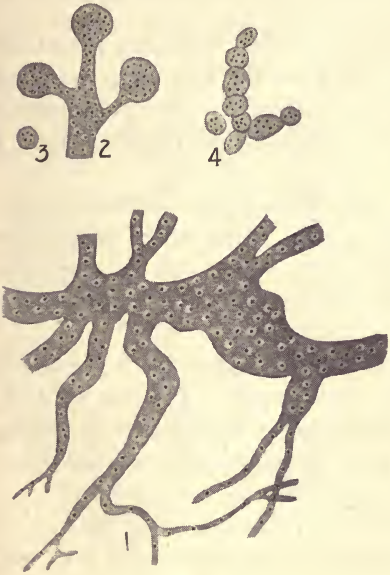

FIG. 24.

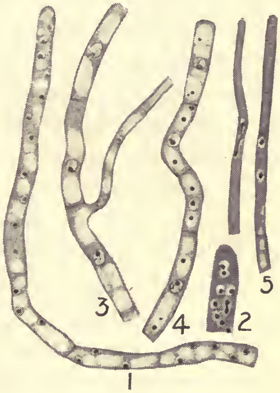

FIG. 25.

FIG. 24.—1, Part of the mycelium of *Thamnidium elegans* (*Mucor*). 2, Extremity of a filament of *Mucor circinelloïdes* showing three swellings about to form sporangia. 3, A spore of the same mold. 4, Yeast forms from the same mold. (*After Léger.*)

FIG. 25.—1, Mycelial filament of *Endomyces magnusii*. 2, Extremity of a filament of the same mold in the process of growth, with a dividing nucleus. 3 and 4, Filaments of *Endomyces fibuliger*. In 4, metachromatic corpuscles are seen in the vacuoles. 5, Filament on the way to increase, from the same mold, the nucleus dividing.

2. Other fungi, especially among the *Ascomycetes*, have a septate mycelium, but one in which the transverse septa do not restrict cellular

functions as true cells. It consists of compartments containing a variable number of nuclei called *coenocytes* (Fig. 25, 1). Each compartment may be considered, not as a true cell, but as a colony of rudimentary cells, energids.

3. Still other molds have a mycelium consisting of true cells with a single nucleus, as for example *Endomyces fibuliger* (Fig. 25, 3 and 4) and *Endomyces decipiens*.

There are, moreover, molds which show both these last two structural types, with transitional forms between the two. For instance, in *Endomyces magnusii*, the mycelium, ordinarily consisting of areas, each containing many energids, can in some parts progress to a uninuclear cellular structure.

The conidia or spores of many molds may have either one or many nuclei, according to the species. The spores of the *Mucorineæ*, for example, always have many nuclei (Fig. 24, 3); on the contrary, the ascospores of the Ascomycetes, the conidia of *Penicillium* and *Aspergillus*, contain generally but a single nucleus.

The yeast forms which result from the budding of the mycelium in some molds, most frequently have a single nucleus (Fig. 24, 4); however, in some, *Dematium*, are sometimes found yeast-forms containing several nuclei. The yeast-forms of the *Mucorineæ*, which are not otherwise very typical forms, are always multinuclear.

To whichever of these three structural forms a mold belongs, it always represents some similar constitutional elements which we will now consider.

CYTOPLASM.—The cytoplasm is a semi-fluid mass, somewhat dense, sometimes homogeneous and containing a more or less considerable number of vacuoles. Certain methods of fixing and staining have recently made possible a demonstration, in the cytoplasm of the most diverse molds, of the presence of a *chondrium*, very clear and always splendidly exhibited. This consists mostly of fine rod-mitochondria, very long and flexible, generally lying parallel with the longitudinal axis of the cell (Fig. 26). Sometimes also it contains granular mitochondria.

The cytoplasm also has reserve products, of which we shall speak later.

NUCLEI.—The nuclei show a differentiated structure which is sometimes difficult to demonstrate. They consist of a nuclear membrane, a hyaline nucleoplasm, a large nucleolus and a chromatic network. The last is sometimes indistinct, and it frequently happens that the nucleus appears to contain only a nucleolus; but a very careful examination always reveals the network (Fig. 25, 3 and 4).

The division of the nucleus is not always easy to observe. To study it, one must examine the growing tips of the mycelium. In some cases this consists in an elongation of the nucleus which soon assumes the form of a very slender dumb-bell which breaks apart at the narrow portion. This is the extent of an amitotic or direct division (Fig. 25, 2 and 5).

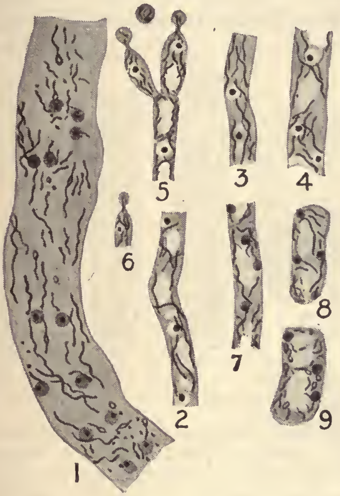

FIG. 26.

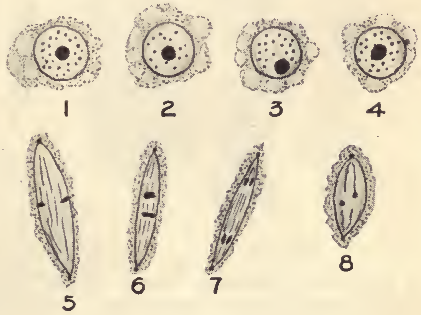

FIG. 27.

FIG. 26.—Various molds fixed and stained by a special technic, showing their chondrium. 1, Filament of *Rhizopus nigricans* (*Mucor*). 2–4, Filaments of *Penicillium glaucum*. 5 and 6, Fragments of the conidial organ of the same mold. 7, Filament of *Endomyces magnusii*. 8 and 9, Oidia of the same mold. In all these molds, chondrium is represented by long filaments, or sometimes by small grains. The filaments often show small vesicles at their crossing.

FIG. 27.—Nucleus of the *Mucor* (1–4), and various stages of its division (5–8). (*After Moreau.*)

Karyokinesis is usually seen only in the organs of fructification (asci, basidia, etc.); nevertheless, in the mycelium of the *Basidiomycetes* and *Mucorineæ*, true métamitoses have been found. In the *Mucorineæ* for example (Fig. 27), the nucleus loses its membrane (1–4) and gives rise to a spindle ending in a centrosome at either extremity, while two chromosomes form the equatorial plate at the center (5). Each of the two chromosomes divides and the four resulting chromosomes are distributed between the two poles (6–8) where they form the two daughter nuclei (Moreau).

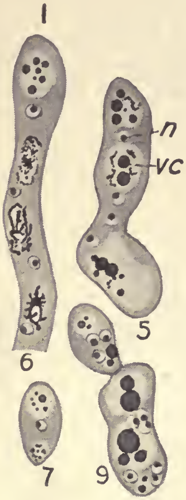

FIG. 28

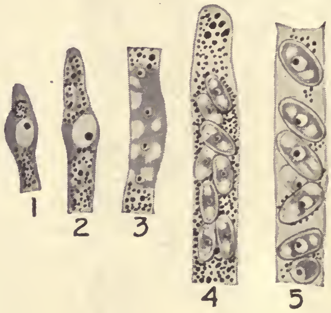

FIG. 29.

FIG. 28—*Dematium* species stained by a method permitting the differentiation of the metachromatic corpuscles. 1, Filament. 7 and 9, Yeast forms. 9, Yeast form starting to bud from mycelium. The metachromatic corpuscles are situated in the vacuoles in the form of small grains joined in chains (6) or isolated. Many appear like large granules (9). *n.* Nucleus. *v.c.* Vacuole with metachromatic corpuscles.

FIG. 29.—Various stages of the development of the ascus in *Aleuria cerea.* 1 and 2, Young asci with their nucleus and many metachromatic corpuscles. 3, Fragments of an ascus after the second nuclear division. 4, Ascus, still young, in which the ascospores are surrounded by metachromatic corpuscles. 5, Older ascus in which most of the metachromatic corpuscles have been absorbed by the ascospores.

METACHROMATIC CORPUSCLES AND RESERVE PRODUCTS.—The vacuoles always contain a great many shining granules, showing Brownian motion and capable of being stained in the living state by neutral red and methylene blue. These bodies have staining qualities which permit them to be easily characterized. They are stained a violet-red by most of the basic dyes, aniline blue or violet. They also take on a very pronounced reddish tinge with hematoxylin (Fig. 28). By reason of this property of *metachromatism*, they have been called *metachromatic corpuscles*. These bodies, which are very common in the *Protista*, have been found in yeasts, bacteria, algæ and protozoa. The chemical nature of the substance constituting them is still unknown, but the name *metachromatin* is often used for it.[1] Some authors, among whom is Arthur Meyer, believe them to consist of a combination of nucleic acid, but this is a mere supposition.

On the other hand, the rôle of the metachromatic corpuscles is now well known. It is evident that they are reserve substances. Their evolution proves it. Thus metachromatic corpuscles appear in great abundance in the young asci of the higher *Ascomycetes* (Fig. 29, 1 and 2), then accumulate in the cytoplasm of the *epiplasm* which is not utilized in the formation of the ascospores, gather all around the ascospores at the time of their forming (3–4), and are gradually absorbed by the latter in the course of their development (5). They therefore furnish nourishment for the ascospores and from this standpoint behave exactly like glycogen and the globules of fat which are usually coëxistent with them in the cytoplasm. We shall see, moreover, that they undergo a similar evolution in the asci of yeasts. Likewise in the conidiophores of molds, notably in the fruiting heads of *Aspergillus* and *Penicillium*, the metachromatic corpuscles are produced in great abundance (Figs. 30 and 31), then gradually disappear as the conidia form (30, 3). Here again they serve as food for the conidia.

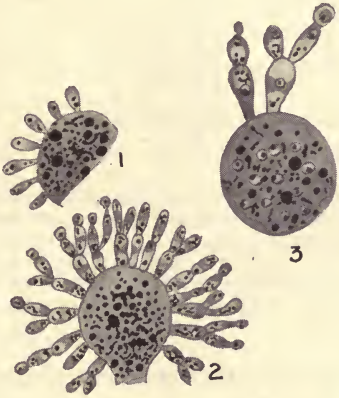

FIG. 30.—Conidial organ of *Aspergillus niger* with metachromatic corpuscles.

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[1] Because of the priority and more exact signification, the names *metachromatic corpuscles* and *metachromatin* are preferable to the terms *grains of volutin* and *volutin* given by Arthur Meyer.

Metachromatic corpuscles appear not only in the vacuoles, but also in the perivacuolar cytoplasm. There they spring up, to diffuse finally in the vacuole where they increase. It is difficult to observe their manner of forming in the mycelial filaments, but in the preparation for sporulation in some molds (asci of the higher *Ascomycetes*), it has recently been demonstrated that they start in the midst of the elements of the chondrium, which act as plastids similar to the plastids of the higher plants. They start in the interior of the granular-mitochondria or in the rod-mitochondria (Fig. 31). In the former case, a small corpuscle appears in the substance of a mitochondrium, then develops gradually, while the mitochondrial membrane which envelops it grows thinner; is reduced to a small capping of the grain on one side; then disappears when the latter reaches maturity. It is noteworthy that the corpuscles emigrate with their plastid to the interior of the vacuoles during their development.

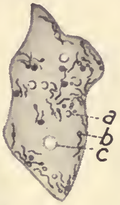

FIG. 31.—Formation of metachromatic corpuscles in a cell of the perithecium of *Pestularia vesiculosa.* The rod-mitochondria form, on their crossings, vesicles (*c*) consisting of a metachromatic corpuscle unstained by the special method which served to differentiate the chondrium. Some corpuscles (*a*), more highly developed, are found in the vacuoles still surrounded by their mitochondrial shell; others (*c*) at the completion of their development have worn through their mitochondrial covering.

When the corpuscles start in a rod-mitochondrium the process is much the same as in the granular-mitochondria; when several rod-mitochondria are involved, at their junction small corpuscles are seen to form; the parts of the rod-mitochondria which join are then absorbed and the corpuscles, enclosed in their mitochondrial membrane, once separated, undergo the same evolution as above.

Thus the metachromatic corpuscles, like grains of starch in the higher plants, start in the midst of the mitochondria and develop gradually out of their mitochondrial matrix with the aid of the vacuolar substance.

In molds are found still other reserve products. One often sees globules of fat in the cytoplasm, which are easily stained by a black-brown by osmic acid; and glycogen which can be differentiated by iodine in iodide of potassium. The glycogen is contained in either the cytoplasm or the vacuoles. It is generally very abundant.

These products (fat and glycogen) undergo the same evolution as the metachromatic corpuscles, and they also accumulate in the organs of fructification (asci, conidial organs) to serve in the nourishment of spores and conidia.

CELL-WALL.—The cell-wall of molds is quite distinct and often thick. It is sometimes cutinized. According to Mangin, it consists of callose and pectose with which is often associated a kind of cellulose.

SPECIFIC CONSIDERATION OF MOLDS*

A few species are found to grow very constantly in the same situations as bacteria. These are associated with forms of decay, fermentation, or disease, either as primary or secondary causes. They thus become important to the bacteriologist who studies them by the same methods as bacteria. These species belong to widely scattered groups of fungi, so that species found under the same conditions frequently differ greatly in appearance. The common term, molds, is applied collectively to these organisms, though no sharp limits can be set to the use of the term. Physiologically these species can be considered in three series:

COSMOPOLITAN SAPROPHYTES.—Certain species are capable of growing within very wide limits of temperature and of composition of substrata. Many of these have accompanied man everywhere and are constantly found upon every kind of putrescible matter, especially as the causes of fermentation or decay in food. Their spores (conidia) are produced in countless numbers, and are so light that they float in air currents and are carried by contact in every conceivable manner by animals and by man. The life cycle from spore to spore is frequently very short, often being completed in twenty-four hours or less. Many of these forms are propagated for an indefinite number of generations by asexual spores or conidia but produce sexual fruit when special conditions are furnished. Some of them have never been induced to develop a

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*Prepared by Charles Thom.

"perfect" form. These species are the "weeds" of the bacterial culture-room, since they cannot be entirely eliminated and often times will survive conditions more severe than the bacteria themselves.

MOLDS OF FERMENTATION.—A few species have acquired special importance by their fermentative action. Certain of these forms are widely distributed and able to utilize other media and conditions. They differ from closely related species of the same genera in the ability to produce special enzymes or especially large amounts of such enzymes as bring about particular forms of fermentation. Certain of these species have been utilized in the manufacture of drinks, of citric acid, in cheese ripening, etc. Others are so adapted to growth under conditions of fermentation as to be found constantly in connection with such processes, in which their vigorous growth and fermenting power seriously interferes with control of results.

PARASITIC MOLDS.—Many species of molds have been described as the cause of diseases in man and domestic animals. A few of these forms have been isolated and studied. Some of them attack the lungs, others the kidneys, but the larger number appear as the cause of diseased areas (dermatomycoses) of the skin, hair follicles, and external ears, or swellings or malformations of the extremities. Of a large number of forms named from a microscopic determination of their presence in particular lesions, only a few have been adequately characterized and shown to be primary agents in causing the injuries observed. *Aspergillus fumigatus*, various species of *Sporotrichum* and *Actinomyces*, the scalp organisms of herpes and favus appear to be real pathogens.

GENERIC CONSIDERATION OF GROUPS*

THE MUCORS OR BLACK MOLDS.—The mucors or black molds constitute a large group of species belonging to the *Phycomycetes* or algal fungi whose general characters are a unicellular mycelium, at least in the vegetative stage, and quite generally a well-developed form of sexual reproduction (Figs. 32 and 33). In the mucors, the mycelium is usually richly developed within and often also on the surface of the substratum; asexual reproduction is accomplished by spores borne as conidia or borne within sporangia; and sexual reproduction is accomplished by the conjugation of special branches from the mycelium forming *zygospores* (Figs. 32 and 33). The typical mucors produce sporangia as capsule-like dilations at the ends of erect fertile hyphæ, each containing many spores. Septa are commonly developed in the mycelium when sporangia begin to appear. These fertile hyphæ may be microscopic or attain a length of several centimeters.

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*The series of forms presented contains representatives of the most common groups as they occur in laboratory cultures, and such as have acquired importance to the worker in bacteriology by participation in processes regularly studied by the bacteriologist. For more complete discussion of the fungi, the student is referred to standard text-books of cryptogamic botany. For discussions of species, Lafar's Technical Mycology includes the groups found associated with the bacteria; for other groups, special botanical literature must be consulted.

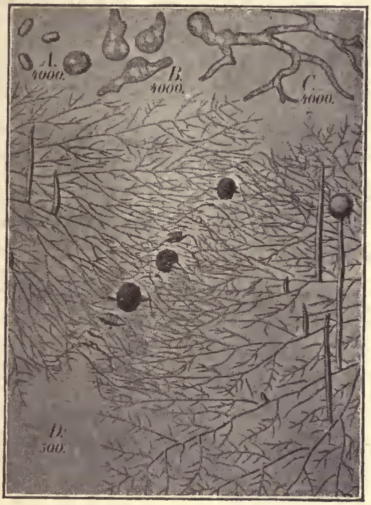

FIG. 32.

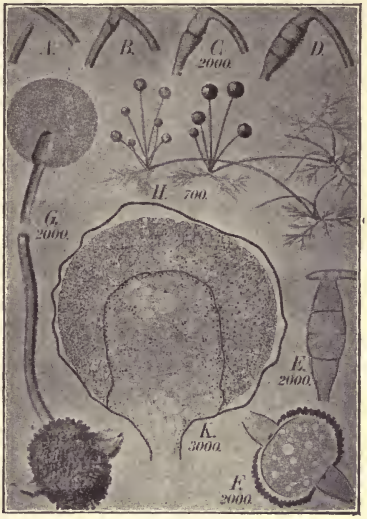

FIG. 33.

FIG. 32.—*Mucorineæ. Mucor. From Tabulæ Botanicæ,* showing sporangia originating from mycelium, spores and spore germination, and the formation of zygospores in a heterothallic species (diagrammatic). (Reduced one-half.) (*By permission of A. F. Blaskeslee.*)

FIG. 33.—*Mucorineæ. Mucor, Rhizopus. A, B, C, D,* Formation of the zygospores from conjugating branches; *E,* section of *D*; *F,* mature zygospores in section; *G,* germination of zygospores; *H,* diagram of fruiting stolons of *Rhizopus nigricans; K,* section of sporangium during spore formation, highly magnified (*From Tabulæ Botanicæ.*) (Reduced one-half.) (*By permission of A. F. Blaskeslee.*)

Important Species.—Perhaps the commonest form is *Rhizopus nigricans* (syn. *Mucor stolonifer*), the black mold of bread, a cosmopolitan species associated with the decay of many kinds of food stored in wet condition or in humid situations. Typical clusters of *sporangiophores* are borne on *stolons* or runners, which are hyphæ extending radially from the center of the colony and fastened to the substratum or to the support at intervals by root-like outgrowths. Abundant growth of this species is found only under very moist conditions or in substrata with high water content. *Rhizopus* is a very common contamination in laboratory cultures.

Many species and races of *Rhizopus* have been described. These have been studied especially in connection with the fermentation industries of Japan and China. Rice, wheat, and soy-beans in various mixtures pass through an initial process of "*Koji*" preparation in which raw or cooked materials are exposed to the air for several days. These processes offer ideal conditions for the entrance of the mucors as contaminations among the organisms desired.

There are many common species of the genus *Mucor*, very few of which are identifiable without critical study. The specific names as commonly cited often designate groups of species or varieties rather than sharply marked forms. Certain of these may be briefly considered.

Mucor mucedo L. is a common form upon dung, characterized by heads (sporangia) upon long sporangiophores,* at first yellow then becoming dark brown or black and studded upon the surface with needles of lime.

Mucor racemosus, Fresenius, is characterized by the production of chlamydospores or cysts in the mycelium within the substratum, as elliptical thick-walled cells. The sporangiophores typically branch to make racemes of sporangia. The racemose mucors are active agents in changing starch to sugar and in the production of traces at least of alcohol from sugars.

Mucor rouxii (Calm.), Wehmer, (syn. *Amylomyces rouxii*) is the most important of a series of forms with sporangiophores branching sympodially which are active in changing starch to sugar and in producing traces at least of alcohol. The mycelium of *Mucor rouxii* develops in fluid cultures as yeast-like cells and groups of cells. The typical mucor fruits are produced only under special cultural conditions. This organism is used in the *amylo process* of alcoholic fermentation.

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* The term *sporangiophore* is composed of the word *sporangium* combined with the suffix *phore*, meaning bearer. In sympodial branching the first fruit is on the tip of the original hypha, the first branch arises below this fruit and is terminated by the second fruit. Each successive branch and fruit originates in similar manner.

Fermentation activity has been described for numerous species of *Mucor* and *Rhizopus*. Among them are *Mucor circinelloides*, Van Tieghem, *Mucor javanicus*, Wehmer, *Mucor plumbeus*, Bonorden, *Rhizopus oryzæ*, Went, *Rhizopus javanicus*. The fermenting power of mucors like that of yeasts varies greatly with the species or even with races used, approaching in some species the efficiency of the more active yeasts.

THAMNIDIUM.—Of related genera, *Thamnidium* differs from *Mucor* in the production of two kinds of sporangia. The terminal sporangium of a fruiting hypha resembles that of *Mucor;* the secondary or accessory sporangia which are borne upon side branches of the sporangiophores are smaller, lack the columella, and produce few to several spores within an outer wall.

Thamnidium elegans, Link, produces primary and secondary sporangia on different hyphæ, together making white colonies. The fertile side branches are produced in whorls and bear whorls of branchlets from their centers which in turn produce sporangioles from the tips of short straight twigs or branchlets.

PENICILLIUM.—The extremely abundant green molds most frequently belong to the genus *Penicillium*, although some members of other groups may be confused with them at times.

Characters.—Colonies are composed of loosely woven hyphæ, branched, septate, colorless, or bright colored. The fertile hyphæ (conidiophores) are mostly erect, arising either from submerged hyphæ, or as branches of aerial hyphæ, septate, usually branched only in the fruiting portion. Conidial fructifications consist of more or less complex systems of branches and branchlets, the ultimate fertile cells each producing a chain of conidia (Fig. 34). The whole system is usually grouped near the end of the conidiophore, giving the appearance of one or more brooms or brushes (whence the name). Very few species are known to produce asci, hence these are rarely encountered. The conidial form continues for an indefinite number of generations, therefore all the activities of the genus are associated with this form. The classification of the whole group, technically transferred by some workers to the ascomycetes on account of certain forms, becomes misleading, because it contains so few species producing asci.

So far as evidence has accumulated nearly all the forms met are imperfect.

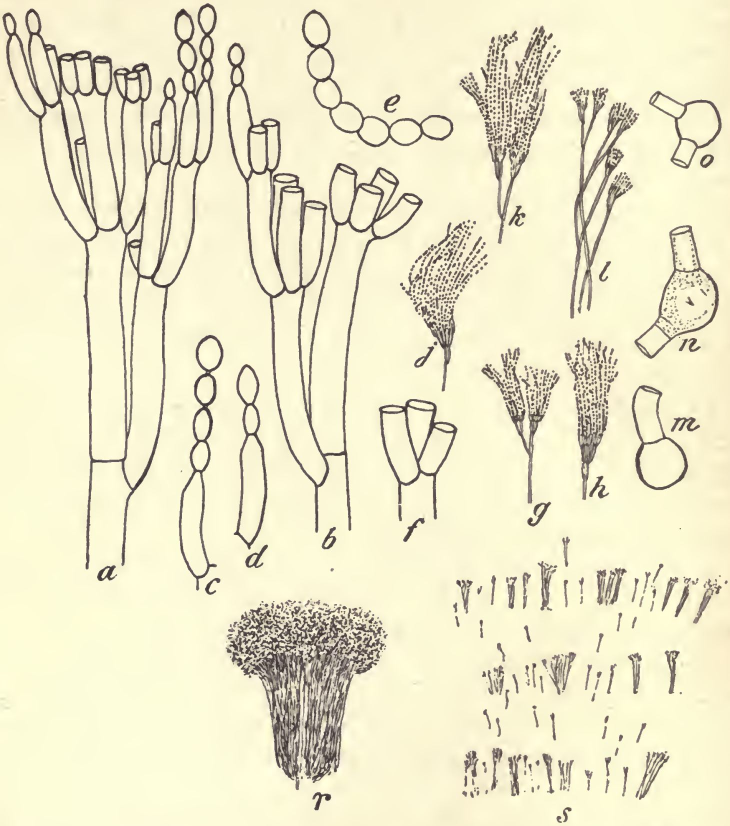

FIG. 34.—*Penicillium expansum*, Link. *a*, *b*, *f*, Branching and arrangement of branches of conidial fructification (×900); *c*, *d*, *e*, conidiiferous cells and conidial chains (×900); *g*, *h*, *j*, *k*, *l*, sketches of fructifications (×140); *m*, *n*, *o*, germination of conidia (×900); *r*, *s*, sketches from photographs showing in *s* loose aggregations of conidiphores beginning to develop into zonately arranged coremia, in *r* a coremium 1 mm. in height. (*From Bul.* 118, *Bureau of Animal Industry, U. S. Dept. Agriculture.)*

Cultural Considerations.—Among the numerous species and races are certain green forms which are widely distributed and almost omnivorous in habit. Other species are closely restricted to particular substrata. Starches and sugars appear to be especially favorable components of nutrient media for members of the group. The larger number of the species grows best at temperatures from 15° to 30°; a very few of them reach their optimum at 37°, but many species are entirely inhibited and some killed at blood-heat. Vegetative mycelium begins to be produced at temperatures very close to freezing, but colored conidia are produced slowly or not at all at low temperatures. The species of *Penicillium* thrive through a wide range of concentration of culture media, though perhaps the most characteristic growths are produced in media high in water content. The common species of this genus grow in all the standard bacteriological media. With few exceptions the species grow well in synthetic media composed of assimilable carbohydrates and inorganic salts. A few species require the presence of some one of the higher nitrogenous compounds, but many species refuse to produce typically colored fruit without some form of starch or sugar in addition to ordinary peptone and beef-extract. Very few species grow well in alkaline media, but most species are tolerant of organic acids at the concentrations found in fruits and vegetables.

Some Common Species.—Penicillium roqueforti, Thom, is a green form constantly found in pure culture in Roquefort cheese, frequently also in ensilage. It is widely distributed and grows under many sets of conditions.

Penicillium camemberti, Thom, is the chief organic agent in ripening Camembert cheese. Cultures of this species are floccose or cottony, at first white, later gray-green.

Penicillium expansum, Link, is a green form, always obtainable from apples decaying in storage, upon which it frequently produces large *coremia* or stalks bearing conidia in masses sometimes several millimeters in diameter. It is one of the most abundant species of the genus, widely distributed in different countries. In cultures, colonies produce a characteristic odor, suggestive of its common habitat, decaying apples.

Penicillium brevicaule, Saccardo, (*Scopulariopsis repens*, Bainier) is a form with rough or spiny brown spores which has been used physiologically to detect the presence of arsenic by its ability to set free arsine from such substrata. A whole series of forms has since been found to possess this character correlated with characteristic spore formation. These species or races are common in the soil both in

Europe and America and appear as frequent contaminations upon cheese and upon cured meats to both of which products they impart peculiarly penetrating ammoniacal flavors. One member of this group has been reported as present in war-wounds.

Except species associated with particular processes or substrata, the identification of the green species of *Penicillium* requires special methods and greater care than is possible aside from special study of the group.

ASPERGILLUS (AND STERIGMATOCYSTIS).—The genus *Aspergillus* includes numerous species which develop under widely different conditions. Many of these forms reach their typical development under drier conditions than *Penicillium* and *Mucor*, such as stored grain, herbarium specimens, dried flesh, or foods containing concentrated sugars, such as jams, jellies, etc. Some excite processes of fermentation, and a few are associated with diseases.

Characters.—The vegetative hyphæ are creeping, submerged in the substratum or sometimes aerial also, branched, septate, usually colorless, and sometimes bright colored. Conidiophores or fertile hyphæ arise by transformation of single hyphal cells into thick-walled and often characteristically shaped foot-cells from which the fertile stalks arise as perpendicular branches which are erect, unseptate, or few-septate, usually much larger in diameter than the vegetative hyphæ, and gradually enlarged upward, ending in more or less abrupt dilations or heads which bear closely packed columnar sterigmata or conidiiferous cells over the whole or a large part of their surface (Fig. 35, *b*). Each of these cells bears, in one group of species, a single chain of conidia; in other species (called by some authorities *Sterigmatocystis*) all or part of these sterigmata bear several secondary sterigmata which bear the conidial chains. Part of the species produce also thin-walled perithecia as variously colored spherical bodies upon the surface of the substrata. These perithecia are filled with eight-spored asci (Fig. 35, *e*). Species in certain groups produce sclerotia instead of perithecia, but many species are not known to produce either perithecia or sclerotia.

Important Species.—Among the species constantly met with, *Aspergillus niger* is recognizable by its black or very dark brown spores and in some strains by black sclerotia. Several black-spored forms are described, but their separation is usually impossible by ordinary methods of culture. *Aspergillus niger* ferments sugar solutions with the production of oxalic acid in considerable quantity. Citric acid fermentation with *Aspergillus niger* has been successfully developed upon a factory basis by Currie in the United States.

Of green forms, *Aspergillus** *glaucus*, Link (*Aspergillus herbariorum*, Wiggers), and *Aspergillus repens*, De Bary, both produce abundant yellow perithecia. These abound upon herbarium specimens, hay, grain, concentrated foods, such as jellies, preserves, dried bread and dried meats upon which they produce green conidial areas which are later dotted with bright yellow perithecia.

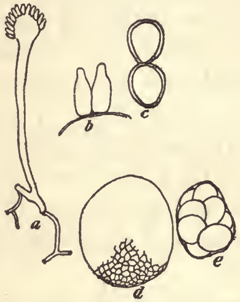

FIG. 35.

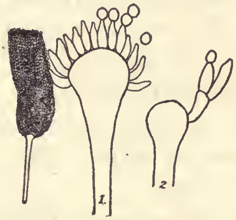

FIG. 36.

FIG. 35.—*Aspergillus glaucus.* *a*, Conidiophore showing increased diameter over the vegetative cells at its base (×128); *b*, sterigmata (×450); *c*, conidia, smooth thick walled in this variety, other varieties are spiny (×450); *d*, perithecium(×128); *e*, ascus containing ascospores (×450). (*Original.*)

FIG. 36.—*Aspergillus.* (1) *A. fumigatus*, Fres; (2) *A. nidulans.* 1 and 2, Show the simple sterigmata of *A. fumigatus* and the secondary sterigmata of *A. nidulans.* The conidia of these species do not remain attached in ordinary fluid mounts. (*Original.*)

Aspergillus fumigatus, Fresenius, is a green form characterized by short conidiophores enlarging gradually into heads and bearing a single set of sterigmata on the very apex, with chains of thin-walled green spores about 3μ† in diameter. This species produces a destructive disease of birds known as aspergillosis. The same species is sometimes reported as pathogenic to man.

Aspergillus nidulans differs by having two sets of sterigmata but otherwise frequently closely resembles *Aspergillus fumigatus*. It is

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*Recent examination of a large number of American specimens shows that *Aspergillus repens* is the usual green form in this country.

†The unit of measurement is the *micron* (μ) or *micro-millimeter* (.001 mm. or 1/25000 in.)

widely distributed in soil. Although it has been reported as pathogenic the identification of this species in pathogenic lesions is not confirmed.

Aspergillus oryzæ and *A. flavus* form a closely intergrading series, certain members of which are used in the fermentation industries of Japan and China. *A. oryzæ* as described by Wehmer is used in the fermentation of rice to produce *Saké*, an alcoholic drink. The diastatic enzyme of *A. oryzæ* grown upon rice converts the starch into sugars which are fermented by yeasts into alcohol. Other members of the series more closely approximating *A. flavus* are widely used in the fermentation of soy-beans. A mixture of cooked soy-beans and cracked roasted wheat is inoculated with *A. flavus* in special *koji* fermenting chambers. In three days the entire mass becomes fully overgrown with mycelium and covered with the ripe conidia of this form. The wheat and beans are partially penetrated by the mold hyphæ. The mass is then transferred to brine strong enough to inhibit further growth except of a few yeasts. In a long period, several months to three years, the whole mass becomes digested to form soy-sauce, or *shoyu.* This product is the basis of meat sauces such as Worcestershire.

Aspergillus wentii, Wehmer, characterized by its long conidiophores and coffee-colored heads of conidia, is found in the *Soja* preparation in Java.

Of other forms constantly met, *Aspergillus candidus* has white or pale cream fruiting surfaces. *A. terreus* is avellaneous in color; *Aspergillus ochraceus*, ocher or tan.

Much confusion is still found in the literature of this genus, so that frequent references to the activities of particular species are difficult or impossible to verify.

MONASCUS.—The organism of red ensilage is widely distributed in the silos of America. Ensilage infected with *Monascus* forms into red balls or masses up to a foot in diameter held together by mycelium. The masses are red from coloring material partly in the mycelium and spore-masses and partly in the silage. The same or a nearly related form described as *Monascus purpureus*, Went, is used in producing red rice, *Ang-quac*, in China. The mycelium penetrates the rice grains, produces a friable texture and gives the whole mass a purple red color. *Ang-quac* (or *Ang-khak*) is used to color Chinese sauces and reaches America especially upon Chinese soy-bean cheeses.

Cladosporium (and Hormodendron).—The species of *Cladosporium* occur frequently in cultures of decaying vegetable matter, of milk and cream, or butter. The colonies liquefy gelatin. Both mycelium and spores are at first colorless, but later dark colored to almost black, with spores becoming two-celled in very old cultures.

Cladosporium herbarum is the commonest species encountered.* Colonies in culture media differ so greatly in structure from those upon natural substrata as to make identification of species questionable. (Fig. 37). Much confusion is therefore found in the use of the names of species of *Cladosporium* and the related genus, *Hormodendron*, which is separated by some.

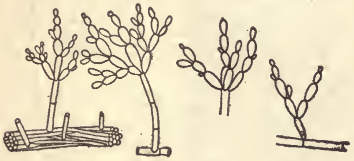

Fig. 37.

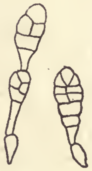

Fig. 38.

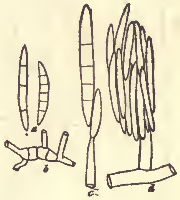

Fig. 39.

Fig. 37.—*Cladosporium herbarum*, showing the forms of conidiophores and conidia which are very common upon laboratory culture media. (*Original.*)

Fig. 38.—Spores of *Alternaria sp.* (*Original.*)

Fig. 39.—*Fusarium* from decaying potato. *a*, Spores showing curvature and septa; *b*, germination of spores; *c*, development of spores in petri-dish culture; *d*, mass of spores as found in culture. (*Original.*)

Alternaria and Fusarium.—The frequent occurrence of species of *Alternaria* and *Fusarium* in cultures demands that the generic characters be recognized. Both, as a rule, produce abundant growth with a tendency to over-run cultures of other forms (Figs. 38, 39). The spores of *Alternaria* are brown, Indian-club form or muriform (divided into several cells by longitudinal as well as cross walls), and are connected together into chains (Fig. 38). The spores of *Fusarium* are colorless, either straight, sickle-shaped, or crescent-shaped, divided into several cells by cross walls, are produced in chains or adhere into masses on the tips of the fertile branchlets. The morphology of colonies in culture varies widely from the descriptions of the same species under natural conditions. Species of *Fusarium* frequently produce bright colors in the mycelium and substrata; colonies of *Alternaria* often become almost black. Identification of species in cultures is thus far impossible, except for the specialist.

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* This species has been shown to be a conidial form of *Spaerella tulasnei* Janczewski, but the bacteriological student will meet only the conidial stage.

OIDIUM.—*Oidium* (*Oospora*) *lactis* is universally found in cultures from milk and milk-products and occurs very frequently in decaying vegetables, manure, etc. Colonies of the species are colorless, have vegetative mycelium entirely submerged, become powdery-white with spores when mature, liquefy gelatin, and produce a strong characteristic odor (Fig. 40). Microscopically the species is recognized by dichotomous branching of the hyphæ at the margin of the rapidly growing colonies, and by the spores or oidia which are abruptly cylindrical, varying with conditions in length and diameter and produced both

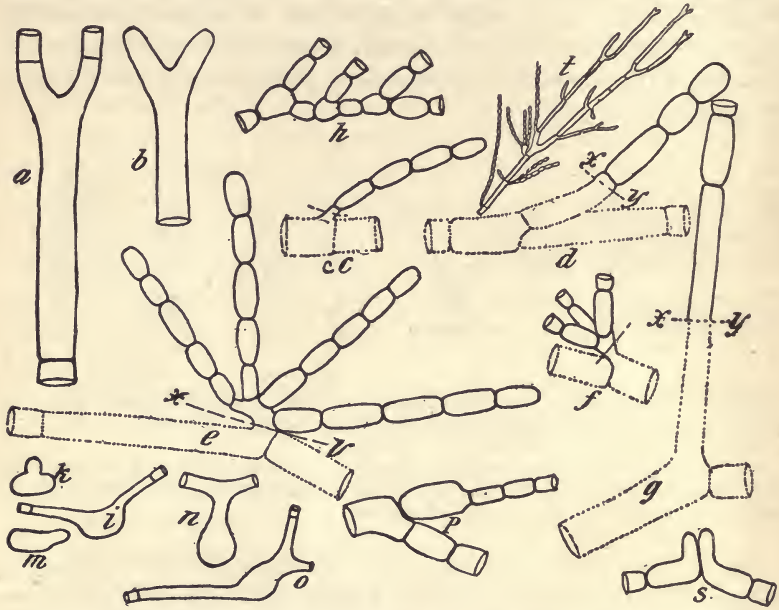

FIG. 40.—*Oidium lactis*. *a, b*, Dichotomous branching of growing hyphæ; *c, d, g*, simple chains of oidia breaking through substratum at dotted line *x–y*, dotted portions submerged; *e, f*, chains of oidia from a branching out-growth of a submerged cell; *h*, branching chain of oidia; *k, l, m, n, o, p, s*, types of germination of oidia under varying conditions; *t*, diagram of a portion of a colony showing habit of *Oidium lactis* as seen in culture media. (*From Bull.* 82, *Bur. Animal Industry, U. S. Dept. Agr.*)

above and below the surface of the substratum in long chains which break up readily. At times the whole mycelium appears to break up into oidia. *Oidium lactis* is a factor in the ripening of many kinds of cheese: Limburger, Harz, Camembert, Gorgonzola, etc., and in the deterioration of butter in storage. Its activity is associated with strong odor and taste.

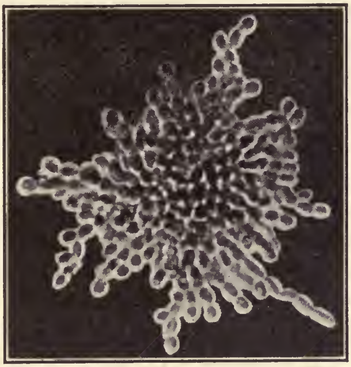

FIG. 41.—A colony of *Monilia candida.* (*Photographed by Z. Northrup.*)

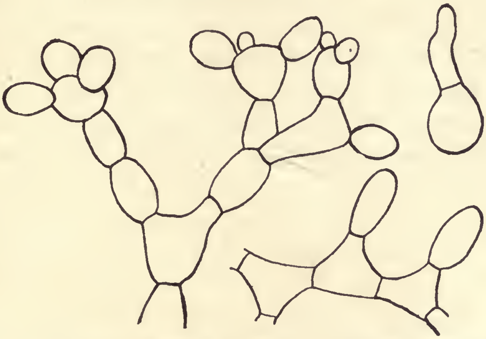

FIG. 42.—Forms of öidia in chains. (*Monilia sitophila.*)

MONILIA.—The generic name *Monilia* is very loosely used in the fermentation literature for certain forms in which the conspicuous development consists of chains of cells like strings of beads. There is a series of borderline forms between the true yeasts, the mycoderma group of yeasts and the true hyphal fungi. The name *Monilia candida* was applied by Hansen to one of these which is found frequently in breweries. The genus *Willia* has been created for another series of these forms. Another widely distributed species, *Monilia sitophila*, forms loose salmon-pink masses of conidia on the surface and in the interior of bread, in cereals and other foods. In culture media *Monilia sitophila* fills culture tubes and dishes with loose fluffy salmon masses of conidia. This organism frequently overruns an incubator or a culture room infecting everything fermentable.

DEMATIUM.—One species of *Dematium*, *Dematium pullulans*, has been much studied. This is frequently found within decaying fruit as dark brown colonies. In culture, mycelium is sparingly produced, either colorless or colored, and conidia are borne in clusters and chains all along the hyphæ submerged in the substratum. At first both mycelium and conidia are colorless, later some or all of the cells develop heavy dark brown walls. Although not active as an agent of fermentation, it occurs very frequently in the fermentation industries sometimes discoloring the fermenting products. The conidia bud out from the cells of the mycelium in a manner resembling the yeasts. Its occurrence with the yeasts has led to many careful descriptions of its several types of spore production and its biological activities.

SAPROLEGNIACEÆ.—This is an aquatic group of *Phycomycetes*, which includes both saprophytes and parasites. Its commonest members grow as shimmering masses of cottony mycelium upon the bodies of flies or other insects in aquaria. Other members of the same group are parasitic, some attacking young fish and producing characteristic lesions. Both sexual and asexual spores (motile swarm spores) are abundantly found.

CHAPTER III

YEASTS*

MORPHOLOGY OF CERTAIN TYPES

DEFINITION AND BASES OF CLASSIFICATION.—If the cloudy freshly expressed juice of grapes or other fruits be passed through a centrifuge, the sediment will be found to consist principally of amorphous particles of dirt and plant tissue. If the clear juice is now allowed to stand in a warm place for a few days it will ferment and the sediment thrown down by the centrifuge may be shown by the microscope to consist principally of unicellular microörganisms.

These microscopic cells are called collectively "yeast" and belong to various groups of fungi. Some of them are special vegetative forms of *Phycomycetes* (*Mucor*), others of *Ascomycetes* (*Saccharomyces, Aspergillus*), while others are unknown in any other form and are classed as *Fungi imperfecti* (*Mycoderma, Torula*). They are widely distributed in nature and some of them occur on all exposed surfaces and particularly on moist organic substances containing sugar and acid. The true yeasts (*Saccharomycetes*), which are of the greatest importance industrially, occur naturally on the raw material (*S. ellipsoideus* on grapes) or are known best in the cultivated condition (*S. cerevisiæ* of beer).

The true yeasts occur in the form of spherical or more or less elongated cells varying in normal width from 2.5μ to 12μ. The first classifications were based on shape and size alone but these vary and depend so much on cultural conditions that they are of little value in differentiating species or varieties.

The range of variation in shape and size, especially of the spores, under given conditions of culture medium and temperature, is now used only in conjunction with the reactions brought about in various solutions to distinguish the various forms.

The true yeasts are characterized by the formation of endospores and are classed with the *Gymnoasceæ*. Each cell seems capable, under favorable conditions, of developing into an ascus. Many unsuccessful attempts have been made to connect the true yeasts genetically with various forms of fungi such as *Mucor, Ustilago* and *Dematium.* At present they must be considered as distinct species.

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*Prepared by F. T. Bioletti. A. Guilliermond has furnished the sections on the "Cytology of Yeasts."

Some yeasts have a tendency during fermentation to remain at the bottom of the liquid; others form a thick foamy layer on top. These are known respectively as *bottom* and *top* yeasts. No sharp distinction can be made as there are intermediate forms.

The vegetative reproduction in the genus *Saccharomyces* takes place by budding, in *Schizosaccharomyces* by fission.

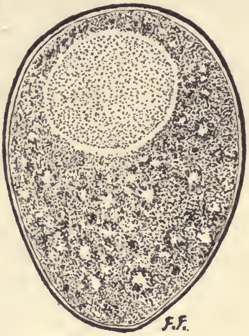

FIG. 43.—Yeast cell. (*Original.*)

The extreme temperatures for budding lie between 1° and 47°, varying with different species. The optimum temperature varies in the same way between 25° and 35°. The rate of multiplication under favorable conditions will range from one to several hours for the formation of a new cell.

When young, vigorous, well-nourished cells are supplied with abundant air and moisture at a comparatively high temperature under conditions that discourage budding (lack of nutriment) they form *endospores.* These spores are usually about half the diameter of the mother cell and from one to eight or more may occur in each cell. They may be formed by cells before or after budding and may even change to asci and form new spores. They are generally spherical or slightly ellipsoidal, rarely kidney-shaped (*S. marxianus*) or furnished with a zonal ring (*S. anomalus*) (Fig. 43).

In nutrient solutions they swell, burst the mother cell, become free and germinate by budding, usually producing vegetative cells directly, though occasionally producing first a short promycelium (*S. ludwigii*).

In *Schizosaccharomyces octosporus* the ascus is formed by the fusion of two cells. Sometimes in other species, two or more spores in one cell will fuse before germination.

Staining with warm carbol-fuchsin and partial decolorization with weak acetic acid leaves the spores red and the cell colorless.

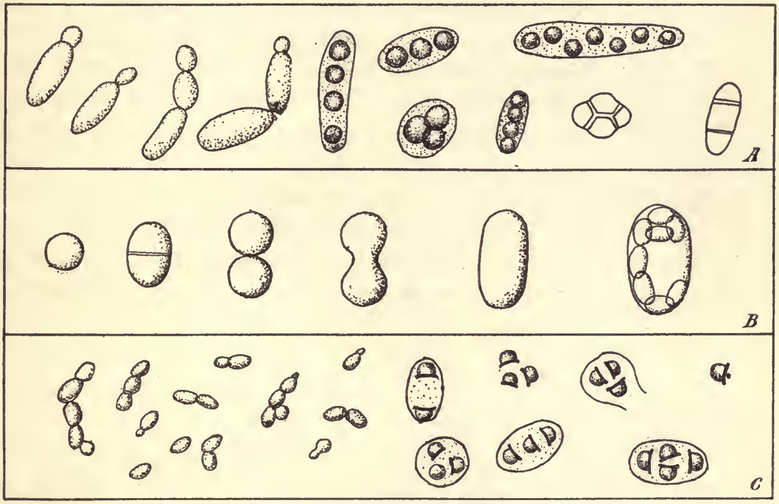

FIG. 44.—Spore-bearing cells. *A*, *S. pasteurianus.* (*After Bioletti.*) *B*, *Sch. octosporus.* (*After Schiönning.*) *C*, *S. anomalus.* (*After Kayser.*)

Cytology of Yeasts*

General Structure of Yeasts.—The structure of yeasts in no way differs from that of the other fungi, only it is seemingly more complex and consequently more difficult to interpret on account of the abundance of the stainable granulations which sometimes accumulate in the cells and occasionally hinder the differentiation of the nucleus. This explains why it has until recently remained a subject of controversy. It is now fairly well understood.

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* Prepared by A. Guilliermond.

In order to understand clearly this structure, one must observe young cells taken from a culture at the beginning of development. For this purpose we use *Saccharomyces cerevisiæ* which, because of the relatively large size of its cells, lends itself better than any other yeast to a cytological study. Examined in the living state, highly magnified, the cells of this yeast show a dense and homogeneous cytoplasm with a group of small vacuoles or a single large vacuole at the center. In the vacuoles and also in the perivacuolar cytoplasm, we can clearly distinguish a great many small shining granules, of varying sizes, which manifest Brownian motion. It is easy to stain them in the living state (Fig. 45) with a very dilute solution of neutral red or methylene blue. These are only metachromatic corpuscles.

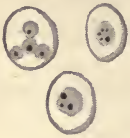

FIG. 45.—*Saccharomyces cerevisiæ.* Young cells examined in the living state in a solution of neutral red. The vacuoles, stained pale red, contain metachromatic corpuscles colored dark red.

In fixed and stained preparations (Fig. 46, 1–10) is seen in each cell a single, large nucleus, whose structure is exactly like that which we have discussed in molds. This nucleus is surrounded by a membrane and contains a hyaline nucleoplasm in which is easily seen a large nucleolus and some chromatin; this latter is scattered through the nucleus, sometimes found in the nucleoplasm in the form of a network, sometimes reduced to a num-

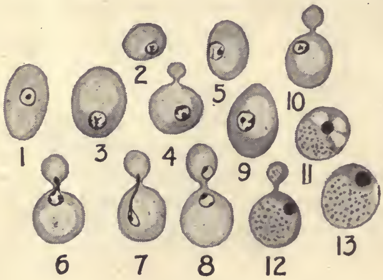

FIG. 46.

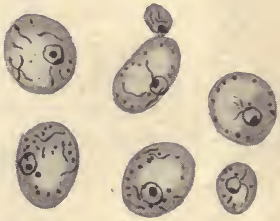

FIG. 47.

FIG. 46.—*Saccharomyces cerevisiæ.* 1–10, Young cells with nucleus, showing its structure. 6–8, The same: division of the nucleus. 11–13, Cells after twenty-four hours' fermentation, with a very large glycogenic vacuole filled with lightly colored grains.

FIG. 47.—*Saccharomyces cerevisiæ.* Young cells fixed and stained by a special method revealing in the cytoplasm a chondrium consisting of rod mitochondria and granular mitochondria.

ber of granules smaller than the nucleolus, and sometimes even found gathered on the circumference of the nuclear membrane.

The cytoplasm is dense and homogeneous. A special technic has recently enabled the demonstration of a chondrium in the cytoplasm. This seems to consist both of granular mitochondria and of more or less elongated and flexible rod-mitochondria (Fig. 47).

The vacuole shows in its interior numerous metachromatic corpuscles of varying sizes (Fig. 48). As in molds, these corpuscles appear not only in the vacuole, but also in the perivacuolar cytoplasm; there they start, and are next diffused in the vacuole where they finish their growth, then dissolve when the need is felt. It is difficult in the case of yeasts to determine their origin; nevertheless, observations made of fungi with larger cells than we have previously described, show that the metachromatic corpuscles start in the midst of mitochondrial elements, and it seems certain that after that the process is the same in yeasts.

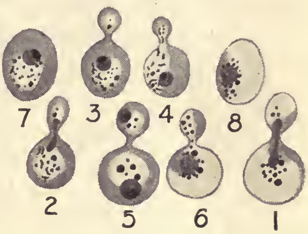

FIG. 48.—*Saccharomyces cerevisiæ*, stained by a method revealing both the nucleus and the metachromatic corpuscles.

In the cytoplasm of yeasts, also, have been noted granulations, which can be stained with ferric hæmatoxylin, and which have been named *basophile grains;* but these formations, which are not well defined, seem to us to represent simply products from the alteration of the chondrium under the influence of imperfect fixing agents.

The membrane of yeasts is quite thick and very distinct. Its chemical nature is still little known. According to some authors, it consists of a cellulose; others think that it contains only pectose. According to Mangin, it is formed of callose. Finally, some authors have thought they discerned chitin.

The structure we have just described is found in all the species (Fig. 49), only it is sometimes much less distinct because of the smallness of the cells. In the elongated yeasts, and in the cells composing the mycelial formation which are encountered under some conditions, especially in the films, the nucleus generally occupies the center of the cell; it is situated in a kind of matrix or bridge consisting of a very dense cytoplasm, while a vacuole filled with metachromatic corpuscles occupies each of the two extremities of the cell.

Summing up, the elements of which a yeast cell consists are a cytoplasm with a chondrium, a nucleus with clearly differentiated structure, vacuoles containing numerous metachromatic corpuscles, a membrane of a nature not yet clearly defined.

Cytological Phenomena during Multiplication.—During the budding of the yeasts, cytoplasm enters the young bud with some chondrium; then, when the bud has reached a certain size, the cytoplasm forms in it a little vacuole in which appear metachromatic corpuscles (Fig. 48, 2–7).

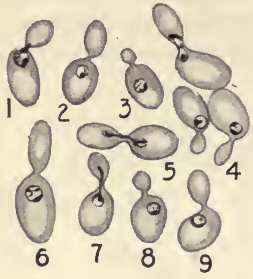

Fig. 49.—*Saccharomyces ellipsoideus.* Young cells each with nucleus.

In the course of these phenomena, the nucleus retains the position which it occupied in the mother cell before the appearance of the bud. Only when the bud is quite large does the nucleus begin to divide. It is elongated so that one end penetrates the bud; the nucleus then resembles an elongated dumb-bell with the larger head remaining in the mother cell and the other, smaller head, in the bud (Fig. 46, 6, 7 and 8; Fig. 48, 2, 7; Fig. 49). Soon the part of the dumb-bell which is stretched out breaks near the neck of the bud, forming two nuclei of unequal size, at first tapering spherical in shape, and later rounded off: one is the nucleus of the mother cell and the other that of the bud. This division is therefore effected by the direct method; it is an *amitosis*. In the *Schizosaccharomyces*, where the cells do not multiply by budding as in other yeasts, but by a transverse partition, the nuclear division is effected by amitosis: the nucleus, situated in the center of the cell, elongates along the longitudinal axis of the cell and resembles a dumb-bell, ending by dividing in the middle, thus forming two nuclei of the same size. Soon a transverse septum appears between the two nuclei and separates the two daughter cells.

We have now to note the modifications which arise in the structure of the cells during the different phases of development and at the time of sporulation.

Variation in the Cellular Structure during Development.—In the course of development, especially during fermentation, yeasts reveal cytological phenomena which render their structure more complex and more difficult to interpret. Let us take for example the study of the *S. cerevisiæ.* After twelve hours of fermentation, the metachromatic corpuscles become more numerous. At the same time, the cytoplasm forms little vacuoles which contain no metachromatic corpuscles, but only glycogen, easily detected by iodo-iodide of potassium. These are gradually fused into a single vacuole, which enlarges much and modifies materially the cell structure. The glycogenic vacuole, increasing, pushes back to the periphery of the cell the cytoplasm, the vacuoles with metachromatic corpuscles, and the nucleus whose chromaticity increases and which becomes homogeneous in appearance (Fig. 46, 11). After forty-eight hours, moreover, the cell is found to consist of an enormous vacuole filled with glycogen which occupies most of it, while the nucleus, the vacuoles with metachromatic corpuscles and the cytoplasm are pushed back to one side of the cell, which is then transformed into a kind of glycogen sack (Fig. 46, 12 and 13; 48, 6–8). At this time the glycogenic vacuole contains a great many small granulations (Fig. 46, 12–13), which easily fix some staining materials, especially ferric hæmatoxylin, and whose origin and significance have not been determined.

Toward the end of fermentation, the glycogen gradually diminishes and the glycogenic vacuole is gradually reduced, then ends by disappearing. The cell after this resumes its original structure.

In the course of these phenomena, the membrane apparently shows no modification. It is known, however, that under some conditions, yeasts secrete gelatinous substances which englobe their cells in a kind of jelly and so appear like zoöglœa (Hansen). It is well to add, on the other hand, that many pathogenic yeasts, when living in the host, have the ability to protect their cells against the reaction of the organisms, by secreting a very thick capsule of gelatinous nature: each of their cells is then surrounded by a large capsule.

CYTOLOGICAL PHENOMENA OF THE SPORULATION AND GERMINATION OF ASCOSPORES.—For a study of the sporulation, we will consider a representative of the species *Schizosaccharomyces*, the *Sch. octosporus*, in which these phenomena are easily observed and especially well understood.

We know that in this yeast, as in some others, sporulation is preceded by a sexual phenomenon consisting of an isogamous copulation. The ascus results from the fusion of two similar cells. The gametes are ordinary cells which have the structure which we have previously described, with one nucleus and one or more metachromatic vacuoles containing corpuscles (Fig. 50, *a*). Fusion takes place between the two cells which are nearest together. Each of these two cells sends out a tiny beak; the two little beaks thus formed anastomose and form a channel of copulation joining the two cells (Fig. 50, *b*, *c*, *d*). The septum separating the two gametes in the middle of the channel is quickly absorbed, and the two cells then have free communication. The cytoplasm of the two cells draws together and mingles in the channel; there the two nuclei draw near to each other (Fig. 50, *e*) and fuse into a single nucleus (Fig. 50, *f*, *g*, *h*). Next the zygote ends its fusion; instead of its original dumb-bell appearance, it assumes the form of an oval cell, then grows large (Fig. 50, *i*). Occasionally, however, it retains a vestige of the individuality of the two gametes, showing two swellings joined by a somewhat narrower middle portion (Fig. 50, *j*).

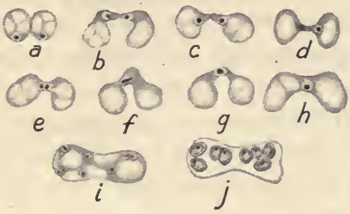

FIG. 50.—Successive stages of copulation and sporulation in *Schizosaccharomyces octosporus*.

During this time, the cell becomes filled with little vacuoles and assumes a more or less alveolar structure. These vacuoles contain a number of metachromatic corpuscles. The nucleus which occupies the center of the zygote begins to divide. The ascus, containing sometimes four, sometimes eight ascospores (Fig. 50, *j*), will then undergo two or three successive divisions, as the case may be. These divisions are accomplished by *karyokinesis* or *mitosis*. In the stages preceding nuclear division, the nucleus is very large and shows a very clear structure with a nucleolus and a chromatic reticulum (Fig. 51, *a*). It soon elongates and assumes a special structure. Its membrane loses its clearness, and in the midst of the nucleoplasm an achromatic spindle appears, ending at each of its two poles in a very small centrosome and containing at its center a group of fine granulations representing the equatorial plate (Fig. 51, *b* and *c*). The

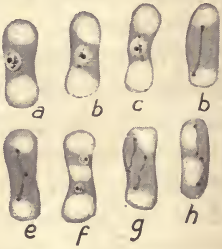

FIG. 51.—*Schizosaccharomyces octosporus*. Various stages of the nuclear division during sporulation.

nucleolus always persists on one side of the spindle. At a subsequent stage the chromatic granulations or chromosomes are divided between the two poles of the spindle, the nucleoplasm is mixed with cytoplasm, then the spindle elongates, while the chromatic granulations form a homogeneous mass at the two poles (Fig. 51 *d, e, g* and *h*). The nucleolus is quickly absorbed, then the two nuclei are formed at the expense of the two chromatic masses (Fig. 51, *f*). To summarize, therefore, this division consists in mesomitoses of a primitive kind, which appear to take place in the interior of the nucleus, whose membrane is absorbed only at the end of the phenomenon. They show the characteristics of the mesomitoses which have been described in the asci of the higher Ascomycetes.

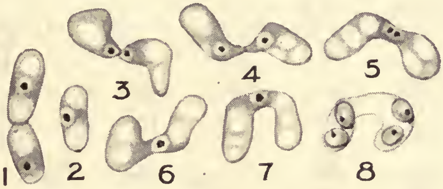

FIG. 52.—Successive stages of copulation and sporulation in *Schizosaccharomyces pombe.* 1–2, Cells just as sporulation is about to begin. 3–7, Union of the two gametes and nuclear fusion. 8, Ripe ascus. Cellular fusion being incomplete, the ascus retains the shape of the two cells joined by a channel of copulation.

When these divisions are accomplished, the nuclei seem to be scattered in the cell (Fig. 50, *i*); they are soon surrounded by a thin layer of cytoplasm which is separated from the cytoplasm by a membrane; these are the ascospores. At first very small, these gradually increase at the expense of the cytoplasm which has not been used in their formation—in other words *epiplasm*—then reach the point where they occupy the whole of the ascus, after having absorbed this epiplasm (Fig. 50, *j*.) The metachromatic corpuscles scattered in the vacuoles of the epiplasm disappear during these phenomena, being absorbed by the ascospores. At no time during the development of the ascus can glycogen be seen any more than in plant cells, but this is replaced by an amyloid substance which is stained blue by iodo-iodide of potassium. This substance impregnates the membrane of the ascospores and disappears during their germination, utilized as a reserve product.

In some *Schizosaccharomyces* or ordinary yeasts which bud (zygosaccharomyces) the ascus comes from an egg which starts in a similar manner (Fig. 52.) In some species, this egg is formed by a heterogamous copulation between an adult cell (macrogamete) and a very young cell which has just separated from the mother cell (microgamete) (Fig. 53). On the contrary, in most species, the ascus results from the simple transformation of an ordinary cell without previous copulation. Whatever may be its origin, the ascus shows cytological phenomena quite similar to those which have just been described in *Sch. octosporus*, with mere differences of detail. Always in *Sch. octosporus* are seen only a few metachromatic corpuscles in the ascus. In most of the other yeasts, on the contrary, the ascus contains a very large number of metachromatic corpuscles, and it is easier there to follow the evolution of these bodies which present interesting singularities clearly demonstrating their rôle as reserve substances.

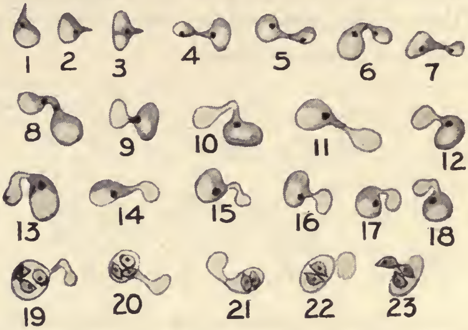

FIG. 53.—Heterogamous copulation in *Zygosaccharomyces chevalieri.* 1–3, Gametes sending out a beak in anticipation of copulation. 4–7, Micro- and macrogametes joined by their channel of copulation. 8, The partition separating the two gametes is absorbed. 9–18, The contents (nucleus and cytoplasm) of the microgamete enter the macrogamete and are fused with the contents of the latter. 19–21, Ripe asci. 22–23, Freeing of the ascospores by rupture of the membrane of the ascus.

Let us observe, for example, the cytological phenomena which appear during sporulation in *Saccharomyces ludwigii.* In this yeast, which shows no sexuality in the origin of the ascus, the cells which are preparing to sporulate assume a finely vacuolar structure (Fig. 54, 8 and 9) and produce a large quantity of reserve products: metachromatic corpuscles, glycogen and fat globules. Metachromatic corpuscles spring up in some vacuoles, glycogen in others; as for the fat globules, they are located in the cytoplasmic web. The nucleus is situated on one side of the cell, surrounded by a thin layer of very thick and homogeneous cytoplasm which is to become the *sporoplasm*, at whose expense the ascospores are formed, the remainder—that is to say the vacuolar cytoplasm—being destined to compose the epiplasm or nourishing plasm.

At a later stage, the metachromatic corpuscles undergo a kind of pulverization transforming them into small grains, and begin to dissolve in the vacuoles surrounding them, the latter at this time taking, with aniline blue stains, a diffuse red coloring similar to that of the metachromatic corpuscles (Fig. 54, 9). At the same time, the nucleus undergoes two successive divisions, but these have not been discernible up to the present time, because of the density and the strong chromaticity of the sporoplasm surrounding the nucleus. They are manifested merely by the appearance of the two daughter cells which migrate to the two poles of the cell, carrying with them a part of the sporoplasm, which assumes the appearance of a dumb-bell and whose

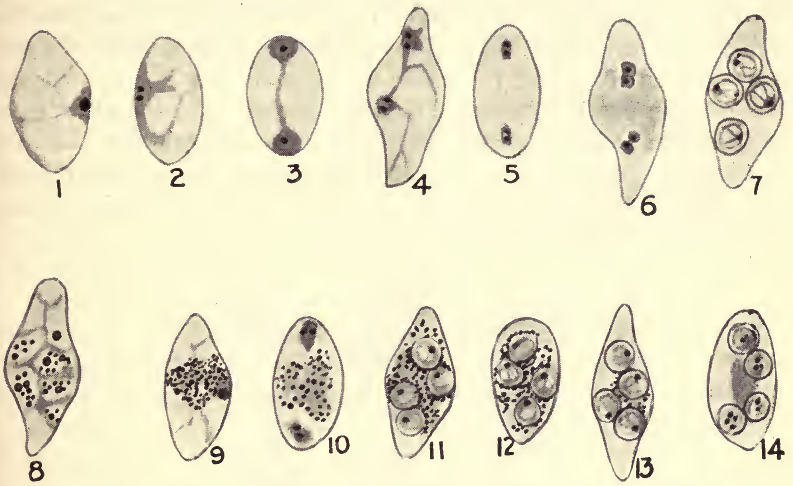

FIG. 54.—Sporulation in *Saccharomyces ludwigii.* Figs. 1 and 7 showing the evolution of the nucleus. Figs. 8–9, the metachromatic corpuscles, stained by a method permitting a differentiation, except in Fig. 8, are dissolving, and the substance of the vacuole which contains them shows a diffuse metachromatic coloring (here gray) like the corpuscles.

slender part ends by breaking (Fig. 54, 2, 3 and 4). The cell, therefore contains at this time at each of its poles a small mass of sporoplasm having first one, then two, nuclei (Fig. 54, 5 and 10). After this, the sporoplasm condenses around each of these nuclei (Fig. 54, 6), thus delimiting at each of the poles two small ascospores.

During these phenomena, the metachromatic corpuscles congregate around the ascospores (Fig. 54, 11 and 12), then gradually dissolve. The ascospores constantly increase in size at the expense of the epiplasm, which becomes disorganized and is reduced to a vacuolar liquid containing in suspension metachromatic corpuscles, fat globules and glycogen. They succeed in absorbing entirely the epiplasm and in occupying the whole of the ascus (Fig. 54, 13 and 14). The metachromatic corpuscles, like the glycogen and the globules of fat, are then completely absorbed by the ascospores, which indicates clearly that they, as well as the latter substances, act as reserve products. When the ascospores are ripe, they contain in their vacuoles metachromatic corpuscles (Fig. 54, 14).

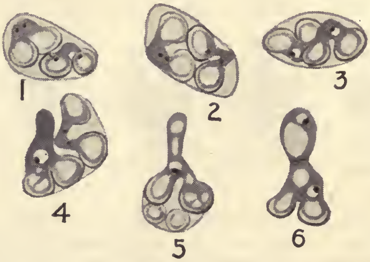

Fig. 55.—Germination of ascospores in *Saccharomyces ludwigii.* 1, Beginning of the fusion of the ascospores. 2, The ascospores are joined two by two by a channel of copulation, but their nuclei are not yet fused. 3, The nuclei are fused. 4, At the left two ascospores, joined, have formed at the middle of the channel of copulation a bud which has ruptured the membrane of the ascus. At the right, the two ascospores, joined by a channel of copulation have not yet fused their nuclei. 5, Formation of the bud at the expense of the two fused ascospores. Two other ascospores have not yet begun their fusion. 6, The bud formed at the channel of copulation is already established and separated from this channel by a transverse septum.

In all yeasts, at the time of budding, the ascospores have the appearance and structure of plant cells. Their germination does not differ from ordinary plant multiplication. In some species, however, especially in *S. ludwigii*, copulation, suppressed at the beginning of sporulation, is replaced by a compensating phenomenon which intervenes at the germination and consists in the fusion of the ascospores two by two (Fig. 55). The ascospores anastomose at their extremities by a channel of copulation which, as soon as the nuclear fusion is accomplished, becomes the seat of a budding.

THE PRINCIPAL YEASTS OF IMPORTANCE TO FERMENTATION INDUSTRIES*

TRUE YEASTS, SACCHAROMYCETES.—The various yeasts used in brewing and some of those used in producing distilling material are grouped together as *S. cerevisiæ.* They are large and round or slightly oval.

They are divided into three main groups—the *bottom yeasts* which are used in the manufacture of German beer, and which, usually, are capable of producing only a moderate amount of alcohol; the *top yeasts,* used in English beers and compressed yeast, capable of producing more alcohol, and the *distillery yeasts,* which have great fermentative power and produce large amounts of alcohol.

Many forms of these yeasts have been described in great detail by Hansen and others but the distinctions are based principally on physiological peculiarities such as the temperature and time limits of film and spore formation, and the character of the fermented liquids. The various forms seem to be fixed, and to retain their characteristics unchanged under almost all forms of treatment.

The wine yeasts, *S. ellipsoideus,* seem to be even more diverse than the beer yeasts, but have been less thoroughly studied. They are somewhat smaller than the latter and usually slightly more elongated. They form spores much more abundantly and easily than the beer yeasts and the cells in film formation are often much elongated.

Their fermentative power is considerable, some of them being capable of producing over 16 per cent by volume of alcohol. W. V. Cruess has obtained 21 per cent from a Burgundy wine yeast. They differ in the flavors and aromas which they produce in the fermented liquid, and especially in the rapidity with which they settle. Some yeasts, such as those of Champagne and Burgundy, form a compact sediment which settles quickly and leaves the liquid clear. Others remain suspended for a long time and settle with difficulty.

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* Prepared by F. T. Bioletti.

Every region seems to have its own forms and the characteristics of the various forms seem to be as well fixed as those of beer yeasts.

Wines are manufactured by the use of these yeasts. They are also employed in distilleries. In breweries they are considered *disease yeasts* and have a deleterious effect on the beer.

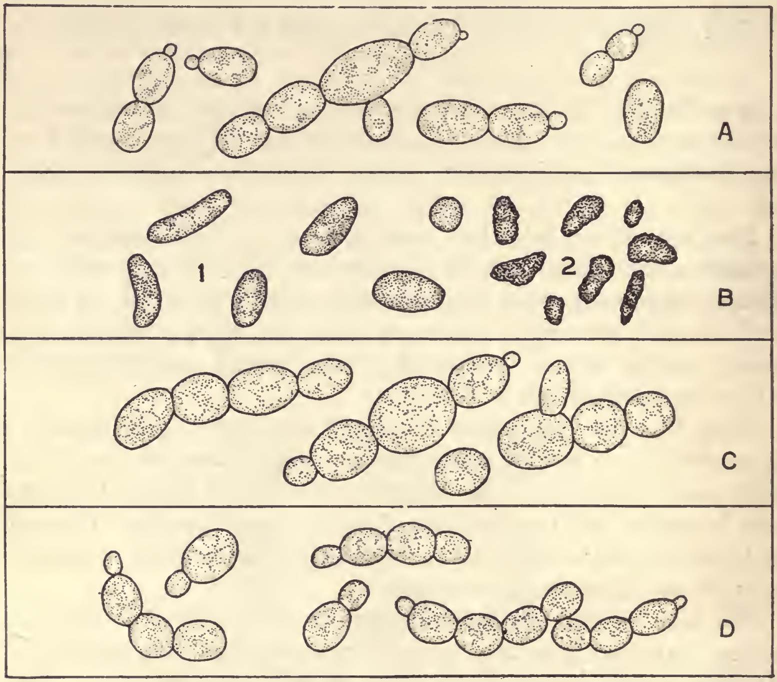

FIG. 56.—Wine and beer yeasts. *A*, *S. ellipsoideus*, young and vigorous; *B*, *S ellipsoideus*, (1) old, (2) dead; *C*, *S. cerevisiæ*, bottom yeast; *D*, *S. cerevisiæ*, top yeast (*Original.*)

S. pyriformis resembles in shape *S. ellipsoideus*, and in association with *Bacterium vermiforme* produces ginger beer.

S. vordermanni is concerned in the manufacture of *arrack*. It ferments the sugar produced from rice by the molds, *Mucor oryzæ* and *Rhizopus oryzæ*.

S. fragilis and other yeasts have been found in kefir and other fermented drinks made from milk. These yeasts working in conjunction with bacteria produce alcoholic acid beverages.

Many true yeasts are more or less injurious. They do not, like bacteria and pseudo-yeasts, cause serious diseases, capable of completely ruining the fermented product, but they may injure the quality more or less. Some yeasts are useful in certain cases and injurious in others. If beer yeasts become contaminated with wine yeast the resulting beer may be persistently turbid. If one attempts to ferment grapes with beer yeast, a wine with a disagreeable beer aroma and of poor keeping qualities is produced.

S. pasteurianus occurs in several forms as an injurious yeast in breweries, causing bitterness and turbidity. Similar forms occur in wine but do little harm except in the absence of the true wine yeast. The cells of this species vary from oval to long ellipsoidal, often being much elongated and in film formation sometimes producing a branching mycelium. Spores are formed easily and abundantly.

The apiculate yeast, *S. apiculatus*, is very abundant on grapes and most acid fruits. It is very variable and undoubtedly includes many varieties. The cells are small, vary in shape from oval to cylindrical, most of them having an apiculation at one or both ends, making them pear or lemon shaped. According to Lindner they form spores in drop cultures, one in a cell. Under favorable conditions this yeast increases with great rapidity, but is checked by 3 to 5 per cent of alcohol. It causes cloudiness in wine, interferes with the growth of the proper yeast and injures the flavor.

Many yeasts, mostly small and some of them rose-colored, have been found on grapes and in wine, but they do not develop under ordinary conditions of wine making sufficiently to be harmful.

Schizosaccharomyces pombe is a yeast found in *pombe* or *millet* beer, made by negroes in Africa. It is cylindrical and large, though variable in size. Both ends are rounded. It multiplies by forming a septum near one end, the smaller division then growing into a normal cell. From one to four spores are formed in a cell. These spores are often produced in the fermenting liquid. The fermentative power is high and a large percentage of alcohol may be formed.

Several other species of this genus have been isolated from grapes and from Jamaica rum.

Pseudo Yeasts.—Budding cells often occur in fermenting liquids which have all the characteristics of yeast except that of producing endospores. They are grouped together under the name of *Torula*.

They are usually small, spherical or slightly elongated. Some species produce a little alcohol and some none. They seldom occur in sufficient quantities to be harmful and one form is accredited with producing the special flavor of some English beers.

The forms included under *Mycoderma* resemble yeast in shape but produce little or no alcohol, are strongly aerobic and do not produce endospores. Their most noticeable characteristic is that they grow only on the surface of the liquid, where they produce a thick film. They cause complete combustion of the alcohol and other organic matters, making beer and wine vapid and finally spoiling them.

Culture of Yeasts

Pure Cultures.—Yeast can be properly studied only in pure cultures. The media used are either the liquids in which the yeasts are to be used such as wort, cider, grape juice, or a special medium devised for a special investigation. An example of the latter is Laurent's medium:

Ammonium sulphate,	4.71 g.
Potassium phosphate,	0.75 g.
Magnesium sulphate,	0.10 g.
Water,	1 L.

To this is to be added any carbohydrate to be studied. Media may be made solid by the addition of gelatin or agar.

Pure cultures can be made, rarely, by inoculation from a naturally pure source, such as the sporangium of a *Mucor*.

Physiological Separation.—The first attempts at purifying mixed cultures were by means of physiological differences. Pasteur freed yeast from bacteria by growing it in a medium containing 2 per cent. of tartaric acid. Effront used fluorides in the same way. These methods may be made more effective by repeated transfers of the culture. Each transfer will contain a larger proportion of the form most suited to the conditions, until finally a pure culture may be obtained. The principle of these methods is of great use in practical fermentation, but is of little use in rigidly separating forms. Methods of general application for the latter purpose must be such that a single cell can be isolated in a sterile medium and a culture propagated from this single cell.

Separation by Dilution in Liquid Media.—A mixed culture is diluted with sterilized water until on the average every two drops contain one cell. A large number of flasks of a sterilized nutrient medium is then inoculated from the dilution, one drop in each flask. If the dilution has been properly made, about half of the flasks will remain sterile and half will show growth. Many or most of the latter will contain pure cultures.

Separation by Dilution in Solid Media.—If we dip a sterilized platinum wire into a mixed culture and then draw it repeatedly over the surface of a solid culture medium such as a slice of sterilized potato or a layer of nutrient gelatin in a petri dish we will get a series of *streak cultures*. The first of these will develop a strong growth of mixed forms. The last will show more and more isolated colonies until some of them will show only a few, some of which may be pure cultures.

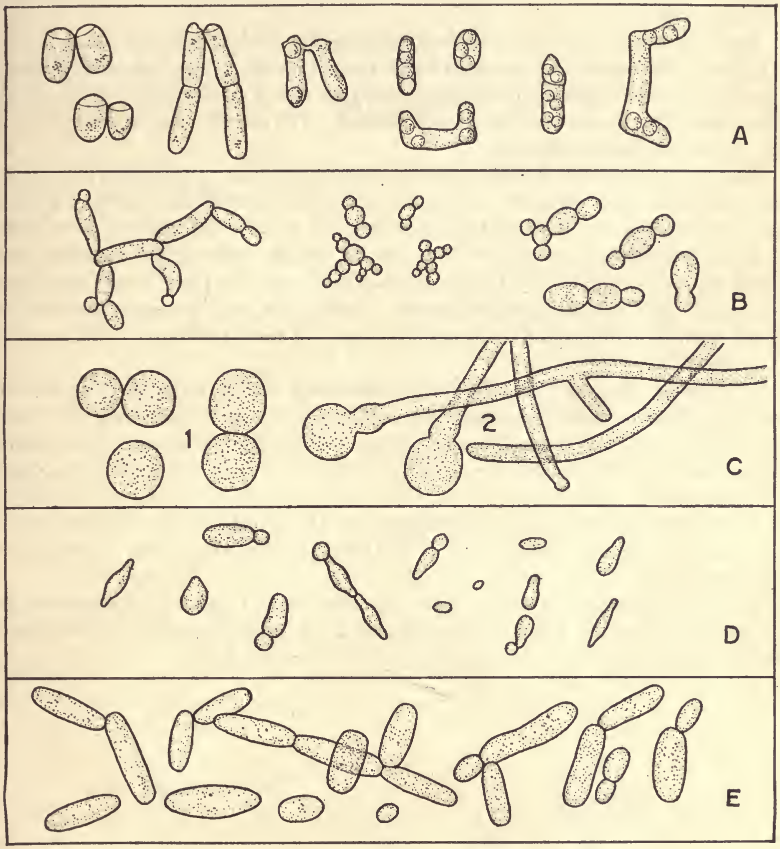

FIG. 57.—Wild and pseudo yeasts. *A*, *S. pombe*. (*After Lindner*). *B*, *Torulæ*. (*After Pasteur*.) *C*, *Mucor*, (1) spores; (2) germinating spores and mycelium. *D*, *S. apiculatus*. *E*, *Mycoderma vini*. (*After Bioletti*.)

The most useful method of separation and one which is applicable to most cases is that of *plate cultures*, first used by Koch and improved by others. In this method a drop of the mixed culture is thoroughly distributed in 10 to 20 c.c. of liquefied nutrient gelatin or agar. A drop of this mixture is then diluted in the same way in another portion of the same medium. This process is continued until the requisite degree of dilution is obtained. The various portions of nutrient gelatin are then poured, with precautions against outside infection, on glass plates or more conveniently into petri dishes. On cooling and solidifying, the gelatin imprisons every cell, each of which on growing gives rise to a colony. It has been found that in practice a small percentage of these colonies may arise from two adhering cells and thus fail to be pure culture.

Hansen's modification of the method is intended to obviate this uncertainty. By making the dilutions in the way described for liquid media, a drop of gelatin containing only one cell is obtained, placed on a cover-glass over a culture slide and, by direct observation, the presence of a single cell verified. The development and multiplication of this cell can be watched.

Differentiation of Yeasts.—With magnifications of 300 to 500, yeast cells can be examined conveniently. Contamination with bacteria and molds of special form can be detected, but otherwise a simple microscopic examination is of little value in determining the purity of a culture. Some information regarding the health, nutrition and vitality of the yeast may be obtained and the form of the spores is of some value in distinguishing species. Yeast cells vary in size as much as in form but under standard conditions each variety will show a certain normal range of dimensions.

If a young, vigorous yeast, in a favorable liquid culture medium, is allowed to remain at rest at a suitable temperature with full access to air and protection from contamination, a growth of cells on the surface will usually take place. This growth may extend over the whole surface (*film formation*) or may be restricted to the edges (*ring formation*). This growth occurs at once with a few species (*S. membranæfaciens*) or at the end of several days (*S. ellipsoideus II*) or may require several weeks. The time and optimum temperature of film formation have been used as descriptive characters.

All the morphological and cultural characteristics of yeast are insufficient for diagnostic purposes and must be supplemented by the physiological characteristics such as their action on various sugars and other carbohydrates.

CHAPTER IV

BACTERIA*

The bacteria naturally fall into quite distinct groups or orders—the true bacteria and the sulphur bacteria.

A portion of the true or *Eubacteria* together with the sulphur forms, are designated as the higher bacteria. The forms usually spoken of as bacteria belong to the group of lower bacteria, and when the word "bacteria" alone is used reference is usually made to the lower bacteria. These constitute a group of microörganisms quite distinct and characteristic, while the higher bacteria form links, as it were, between the lower bacteria and other closely related microörganisms. The morphology of the two groups will need to be discussed separately.*

FORMS OF LOWER BACTERIA*

FUNDAMENTAL FORM TYPES.—The forms of bacteria are exceedingly simple. They are either spheres, straight rods, or bent rods (spiral). In the spherical form they are known as *cocci*, or *micrococci* (sing. *coccus* or *micrococcus*). The straight rods are *bacilli* (sing. *bacillus*) and the bent rods are *spirilla* (sing. *spirillum*).

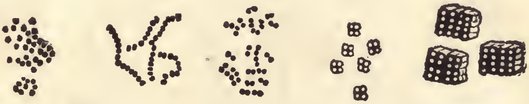

FIG. 58.—Types of micrococci. (*After Williams.*)

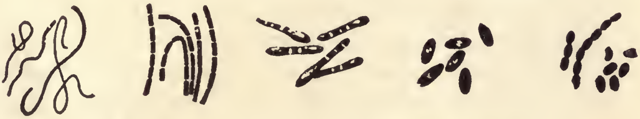

FIG. 59.—Types of bacilli. (*After Williams.*)

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*Prepared by W. D. Frost, with cytology by A. Guilliermond.

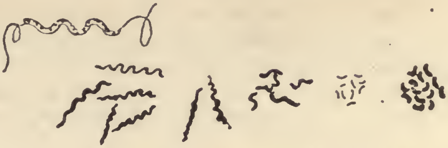

FIG. 60.—Types of spirilla. (*After Williams.*)

GRADATIONS.—The difference between these fundamental form types is frequently very slight. It becomes a very difficult matter, for instance, to distinguish at times between the micrococcus and the bacillus. There is a number of bacteria, and among them the well-known example of *B. prodigiosus*, which are described at one time by one investigator as micrococci and at another time, or, by another investigator, as bacilli. The pneumonia germ is also another illustration of an organism that occupies a dual position. Migula has suggested a method of differentiating these which will be discussed under a later head. The bacilli pass almost imperceptibly into the spirilla. The cholera bacillus of Koch is in reality a spirillum.

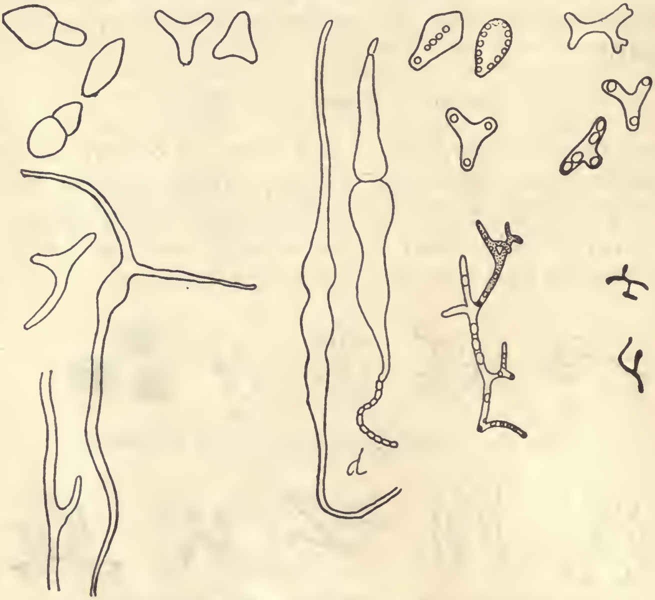

FIG. 61.—*Involution forms.* Here are illustrated unusual forms of *B. subtilis*, water bacteria, *Bact. aceti*, *Bact. pasteurianum*, bacteroids in root nodules, *Bact. tuberculosis*, *Bact. diphtheriæ*. (*After Fischer from Frost and McCampbell.*)

INVOLUTION FORMS.*—The forms of bacteria are quite constant under normal conditions, but very frequently they show abnormal or bizarre shapes. These are known as involution forms (Fig. 61). It is sometimes suggested that these involution forms represent another stage in the developmental history of the organism, and upon this supposition certain bacteria which very regularly show these involution forms have been classified as belonging to a different suborder from that in which the lower bacteria are placed. The ordinary view of the involution forms is, however, that they are degeneration forms, that they correspond, in other words, to the halt and maimed in society and are to be accounted for by the fact that they are deformed by their own by-products. In fact, it is quite probable that they are autogenic. Involution forms are very likely to occur in artificial culture and are much more common with some species than with others. (See page 100.)

SIZE*

The bacteria were formerly spoken of as the smallest of living things, but since the recognition of the ultramicroscopic organisms it is necessary to be somewhat more specific in characterizing their dimensions. The unit of measurement in microscopy is the micron (μ), or micromillimeter. This is .001 mm. or approximately 1/25000 of an inch. Applying this unit to the bacteria we find that the micrococci and the short diameter of the bacilli and spirilla average about 1μ. The micrococci vary in diameter from a small fraction of a micron to three or four microns in diameter. The bacilli are sometimes very small, as the influenza bacterium with a width of 0.2μ and a length of 0.5μ, and sometimes very large as, for example, the *Bact. anthracis* with a width of 1.2μ and a length of 5.20μ. The spirilla average about 1.0μ in diameter but may be as long as 30μ–40μ.

MOTILITY*

When bacteria are viewed under the microscope in a living condition many of them are seen to move. This movement may be one of two kinds. In some cases it is progressive, the individuals move about from one part of the field of the microscope to another and change their relative positions. In other cases the movement is vibratory, the bacteria move back and forth and rotate but do not progress or change their relative positions to any extent. This latter form of movement is known as *brownian movement*, because it was first described by Brown.

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*Prepared by W. D. Frost.

Brownian Movement.—This movement is probably caused by the impact of the molecules of the suspending medium and for this reason is sometimes called molecular movement. It is not characteristic of bacteria, or indeed of life, but is shared by many small microscopical objects when suspended in a fluid medium. Most beautiful examples of brownian movement can be seen by suspending granules of India ink or carmine and examining them under the microscope. This brownian movement is to be sharply differentiated from *vital movement* which is possessed by some bacteria.

Vital Movement.—As already indicated, bacteria have the power of independent movement due to inherent vital power. Only a few of the micrococci are motile, while many of the bacilli and spirilla are. This movement is a change of position and is caused by certain protoplasmic processes which these bacteria possess, known as *cilia* (sing. *cilium*) or *flagella* (sing. *flagellum*). The fact of motility or non-motility of an organism is of considerable value to the systematist. It is determined by examination in a *hanging drop*. At times, however, it varies so little from the brownian movement that it is difficult to tell whether a particular organism or culture does or does not possess vital movement. An opinion can be more definitely formed at times if some chemical producing an anæsthetizing effect on the bacteria is introduced into the examining medium. In case the organism is actually motile its movement will be altered by the anæsthetic but in case it is merely a brownian movement there will be no change.

Organs of Locomotion.—The protoplasmic threads referred to as the organs of locomotion are known as flagella, or cilia. The difference between the cilium and flagellum is the fact that a cilium has a simple curve while a flagellum has a compound curve, like a whip lash. Most of the bacteria possess flagella rather than cilia. The size, arrangement, etc., of these flagella are constant and characteristic of a particular organism. Their structure and arrangement, therefore, will be discussed later.

Character of Movement.—Different bacteria exhibit different kinds of movement. Some dart forward with great rapidity, others move slowly; some move in straight lines, others wobble, but any particular character is quite constant and many of the bacteria may be recognized by their peculiar movements.

Rate.—The rate at which the bacteria travel when they possess vital movement varies greatly. Some of them move very fast, others very slowly. Many of them appear to move with wonderful rapidity. Van Leeuwenhoek, when he first saw these moving bacteria, said that they traveled with such great rapidity that they tore through one another, but it must be borne in mind that under the high powers of the microscope the rate of movement is magnified to the same extent as the object, and that in reality the rate of movement is not excessive. When compared to their size, the rate of movement is probably little greater than that of a trotting horse and considerably less than that of a speeding automobile or a railroad train.

Reproduction*

Reproduction among the bacteria is largely asexual and takes place ordinarily by what is known as binary fission. In addition to this a number of bacteria go into a resting stage, or produce spores. The spore formation is not, however, a method of multiplication, because usually only a single spore is formed in a cell, but serves to tide the organism through unfavorable conditions.

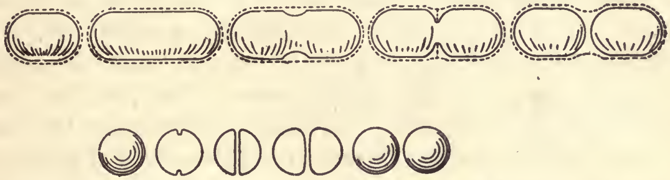


Fig. 62.—The division of bacterial cells (diagrammatic). (*After Novy.*)

Vegetative Multiplication.—This is accomplished by means of binary fission (Fig. 62). When a bacterium has reached maturity, fission begins. Division begins by an invagination of the protoplasm in the middle of the cell, which proceeds until the cell protoplasm is completely separated. The cell wall then grows in and finally splits forming the two ends of the new cells. These new cell walls are formed at right angles with the long axis of the cell in the case of the bacilli and spirilla, except in rare instances. In the case of micrococci, the throwing of the cell wall across one diameter is quite as economical as any other and may therefore proceed in any direction. Migula makes a considerable point of the fact that bacilli and spirilla elongate before division and micrococci divide before they elongate; this

* Prepared by W. D. Frost.

would be the criterion which he would use to separate these two form types. A generation among the bacteria is from one division of the cell to another. This is sometimes very short, in fact, only twenty to thirty minutes. Many of the bacteria after half-an-hour's time have grown from newly formed cells to maturity and are ready to divide again. This makes it possible for bacteria to multiply with very great rapidity, and if we know the length of the generation in a particular bacterium it would be easy enough to estimate the rate of multiplication, at least theoretically. It would be only a matter of geometrical progression. It is of course quite impossible for the bacteria to maintain their theoretical rate of growth for any length of time, but, practically, they grow with enormous rapidity, as is shown in cultures and by the changes which they bring about in nature, such as the production of fermentation and the generation of toxin. Four periods in the life history have been described. A latent or lag period, which is the time elapsing between the seeding and the time at which the maximum rate of growth begins; the logarithmic period or the time when the rate of growth is at its maximum; a stationary period when the increase becomes less and less and finally ceases; and the period of decline when the organisms begin to die.

Spore Formation.—A considerable number of bacteria form spores within the cell. Because they are formed within the cell they are spoken of as *endospores*. Endospores are formed by the bacilli and the spirilla, but not by the micrococci. Their chief value to the cell is their ability to resist unusual conditions, and to enable the individuals of a species to pass through unfavorable conditions which to the ordinary vegetative form of the cell would prove disastrous. At the maturity of the cell, spore formation may begin. It is an open question whether spore formation occurs as a regular stage in the life history of an organism, or is produced only under the stimulus of unfavorable environmental conditions. Both theories have their advocates. The first evidence of spore formation in the cell is a granulation of the protoplasm of the cell. As spore formation proceeds the granules become larger and collect at one portion of the cell. These granules then fuse to form the spore, which soon surrounds itself with a spore wall. At times the spore is smaller than the mother cell and is formed without changing the shape of the cell. At other times it is larger than the mother cell and causes a bulging of the latter. The position of the spore in the cell varies (Fig. 64). In some species it is *equatorial*, in others it is *polar*, and in still others it has an *intermediate* position between equatorial and polar. When the spore is larger than the mother cell and is situated equatorially it causes the cell to bulge with the formation of a barrel-shaped organism, a *clostridium*. If the spore is situated at the poles and is larger than the mother cell, a *capitate* or *drum-stick* bacillus is produced. When the spore is smaller than the mother cell and the cells form in chains, there is frequently a tendency for the spores to be formed in opposite ends of contiguous cells of the chain so that they appear in pairs. The reason for this is not understood. When the spore has reached maturity, the mother cell disintegrates and finally disappears, leaving the endospore free.

The endospores possess remarkable powers of resistance due to the concentrated character of the protoplasm, or to the character of the spore wall. The resistance here may be due to the structure of the wall itself or to the chemical substances which it contains. It is readily conceivable that the presence of certain fatty acids, or higher alcohols, might give the spore its remarkable resistance. These spores are very resistant to desiccation; they have been preserved in a dried condition for many years. They are also very resistant to the action of heat; some forms are known to withstand a temperature of boiling water for as long a time even as sixteen hours. They are resistant also to chemicals and the action of sunlight, although in some cases, as pointed out by Marshall Ward, the very chemical substances which furnish them the powers of resistance toward environmental factors may be broken up under the influence of sunlight, forming poisons so that the spore is killed more readily than the vegetative cell would be.

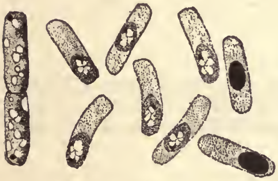


FIG. 63.

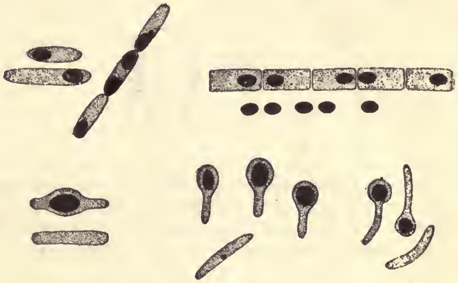


FIG. 64.

FIG. 63.—The formation of spores. (*After Fischer from Frost and McCampbell.*)
FIG. 64.—Spores and their location in bacterial cells. (*After Frost and McCampbell.*)

When these spores are brought under favorable conditions of moisture, temperature, and food supply, they germinate. There are several types of germination (Fig. 65). In some cases the spore wall ruptures at the pole and the young cell emerges so that its long axis is in the same direction as the long axis of the spore. In another type the spore ruptures equatorially and the young cell emerges with its long axis at right angles to the long axis of the spore. In still another type the spore swells and the young cell absorbs the wall of the spore.

In the lower bacteria only a single spore is formed in a cell. In the case of the higher bacteria, however, a number of spores may be formed at the distal end of the filament. These are spoken of as *conidia*, and possess properties similar to those of the endospores.

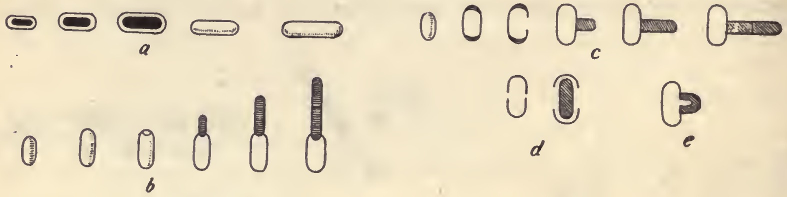


FIG. 65.—*Spore germination.* *a*, Direct conversion of a spore into a bacillus without the shedding of a spore-wall (*B. leptosporus*); *b*, polar germination of *Bact. anthracis; c*, equatorial germination of *B. subtilis; d*, same of *B. megatherium; e*, same with "horse-shoe" presentation. (*After Novy.*)

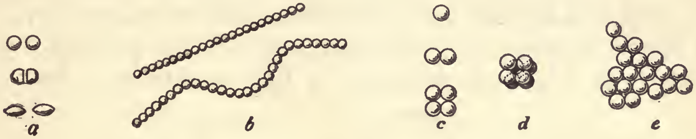
In some cultures of bacteria, as for example in the micrococci, certain cells seem to be larger and different from the other cells. In a streptococcus filament, certain cells suggest to the observer the *joint spores* of the algæ and have therefore been spoken of as *arthrospores* or *joint spores.* There is, however, no evidence of an experimental nature, which warrants the belief that these cells are in reality spores, and it must be said that at the present time the presence of arthrospores among the bacteria is purely hypothetical.

CELL GROUPING*

Bacteria rarely occur singly but usually in groups. These cell aggregates are frequently very constant and quite characteristic of the organism possessing them. They are of sufficient definiteness and constancy to be used by the systematists in characterizing large groups.

* Prepared by W. D. Frost.

Cell Aggregates among the Micrococci.—The grouping of micrococci depends upon the plane of division and also upon the cohesion of the cells. Since it is quite as economical for the micrococcus to divide in one direction as another, it is possible for a number of different cell groupings to occur. Whatever the direction of the dividing walls, it is usually quite constant; if a particular species of micrococci has its planes of division parallel, there will be formed chains of micrococci. In some cases the cohesion is slight and only two cells remain attached to each other, forming what are ordinarily known as *diplococci.* There is a considerable number of very well-known bacteria that are diplococci (Fig. 66). If the cohesion is stronger, we have chains of micrococci or rosaries formed which are known as *streptococci.* Well-known and very important bacteria are grouped in this way. In other micrococci the cell wall is not formed continuously in parallel planes but in



clumped with no apparent geomet form

Fig. 66.—Division forms of micrococci. *a, Diplococcus,* perfect form with flattened opposed surface (*gonococcus*), lanceolate form (*pneumococcus*); *b, streptococcus; c,* consecutive fission yielding a *tetrad; d, sarcina* form resulting from division of tetrad *c; e, staphylococcus.* (*After Novy.*)

planes which alternate at right angles to each other. In this way cell aggregates occupying two dimensions of space are formed. These are known as *tetracocci,* or *merismopedia.* Still again, the planes of division may proceed at right angles to each other in three dimensions of space. In this case packets are formed which are known as *packet cocci,* or *sarcinæ.* Another group of the micrococci occurs, known as the *staphylococci,* so called because they are arranged in irregular bunches, like a bunch of grapes. This arrangement may be due to the fact that these micrococci divide in many different planes, or because during the course of their growth their arrangement is changed.

Cell Aggregates among the Bacilli.—In the case of the bacilli, one diameter is usually considerably shorter than the other, so that nature almost invariably throws the new cell wall across the bacilli at right angles to their long axis (Fig. 67). There is, therefore, only one arrangement or cell grouping possible, and that is end to end, so that *streptobacilli* are formed. When arranged in pairs, the designation is *diplobacilli.* The length of the chains appears to depend not only upon the cohesion of the bacilli but also upon the shape of the end; those which have square ends frequently have very long chains, while those with rounded ends have short chains or occur singly.

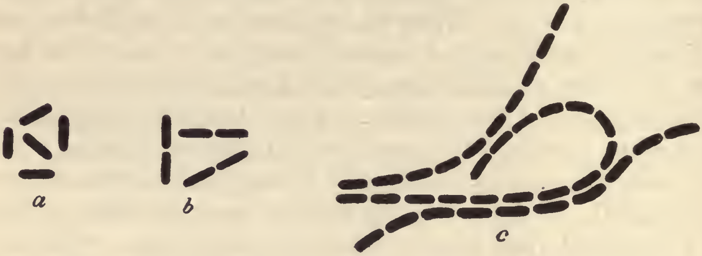


FIG. 67.—Division forms of bacilli. *a*, Single; *b*, pairs; *c*, in threads. (*After Novy.*)

A unique growth-form or cell aggregate is that due to the post fission movement of the cell as described by Hill in cultures of *Bact. diphtheriae.* On fission the two daughter cells are not completely separated but remain attached at one place. This leads to a movement similar to the closing of a jack knife. In this way the two sister cells are brought to rest at an obtuse, a right or an acute angle to each other. They may be even brought parallel.

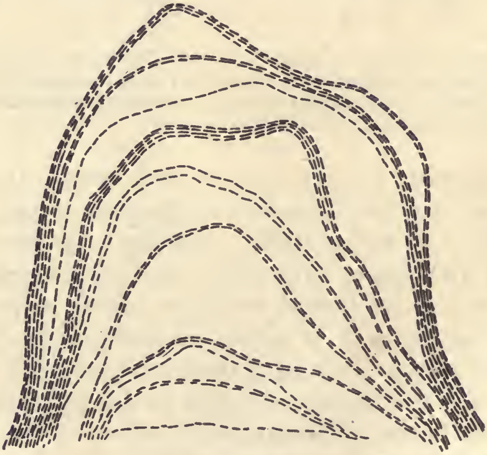


FIG. 68.—Threads of *Bact. anthracis.* (*After Migula.*)

Cell Aggregates among the Spirilla.—The same kind of arrangement is maintained among the spirilla.

Zoogl&oelig;a.—Some of the bacteria secrete a mucilaginous substance which causes the cohesion of the cells frequently in considerable number. This aggregate of cells may assume some characteristic appearance and a great many attempts have been made by systematists to make use of this in differentiating species. These zoogl&oelig;ic masses usually assume the forms of pellicles, but their value as diagnostic features is not great. The formation of zoogl&oelig;a is very frequently only a stage in the life history of an organism.

The Cytology of Bacteria

* The typical cell, such as that of a higher plant or animal, is made up of cytoplasm surrounded by a cell wall. The cytoplasm contains a nucleus. There are also frequently present other evidences of structure in the cytoplasm, such as nucleolus, polar bodies, etc. In addition to these there may be appendages; such as the cilia or flagella. In the case of bacterial cells, we find most of these structures present, such as cell wall, cytoplasm, and appendages.

General Consideration of Cytoplasm and Nucleus.*—The cytoplasm of the bacterial cell is similar to the cytoplasm of other cells except that chemical analyses seem to show that it contains a higher percentage of nitrogen. As viewed under the microscope, in either an unstained or stained condition, it appears as a homogeneous mass filling the entire cell and rarely showing any evidence of structure. Ordinary stains, such as are used in animal and plant histology, fail to reveal the presence of a nucleus, the whole cell being usually uniformly stained with those stains generally characterized as nuclear stains. When these stains are applied to some bacteria, particularly at certain stages of their growth, certain parts stain more readily than others, and we get either what is known as a bi-polar stain or polar

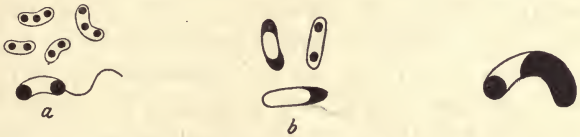


Fig. 69.—Plasmolytic changes. (*After A. Fischer.*) *a*, Cholera vibrio; *b*, typhoid bacillus; *c*, *Spirillum undula*. (*From Novy.*)

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* Prepared by W. D. Frost.

granules. In the first case, the ends of bacilli are stained more deeply than the center so that the cells appear very much as diplococci. This bi-polar stain is characteristic of such organisms as the bacterium of chicken cholera or the bacterium of bubonic plague. The polar granules are frequently seen in the diphtheria bacterium and may be located at the poles and also at the center. In this germ and in some others it is possible, by special staining, to give the granules a different color from the rest of the organism. In this case these bodies are spoken of as *metachromatic granules* which are considered later under "Reserve Products." The presence of these granules might possibly be explained upon the theory that the cells are plasmolyzed (Fig. 69). As a result of plasmolysis the protoplasm of the cell is drawn away from the cell wall and concentrated in areas which would very well explain the appearances. And it seems likely also that the methods employed in staining might lead to plasmolysis, but the metachromatic granules can hardly be explained upon this supposition.

The cytoplasm of the bacterial cell is slightly refractive. It is colorless except in a few cases in which the green coloring matter, like chlorophyl, is present, as, for instance, *Bact. viride* and *Bact. chlorinum.* In the purple sulphur bacteria, the coloring matter *bacteriopurpurin* is present. The bacterial cytoplasm contains vacuoles at times.

Minute Considerations of Cytoplasm and Nucleus.*—The question of the cytology of bacteria has long excited the curiosity of biologists. It is indeed of great importance from many points of view. In the first place, we are interested to know whether bacteria are ordinary cells having a nucleus; or whether, as some maintain, they lack entirely a nuclear element and are an exception to the rule elsewhere established. Moreover, the cytologic study of bacteria may furnish useful knowledge concerning the phylogeny and taxonomy of these organisms, a matter not yet solved. Finally, we may hope that it will throw light upon some problems of a physiological or pathological nature.

Unfortunately this study is very delicate, because of the extreme minuteness of the bacterial cells, so that in spite of the large number of researches which it has incited in the last twenty-five years, it is to this day a matter of controversy.

At present three theories are held by authors relative to the interpretation of the general structure of bacteria. We will examine these three theories one by one, endeavoring to determine which one, in our opinion, seems most probable.

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* **Prepared by A. Guilliermond.**

One of these theories claims that bacteria are cells of very primitive organization lacking nucleus and consisting simply of cytoplasm with vacuoles. The cytoplasm contains many stainable granulations, but these represent products of nutrition. Such an opinion scarcely accords with our knowledge of the constitution of the other *Protista*, in all of which the existence of a typical nucleus, or at least of chromatic elements replacing the nucleus, has been established. This view has not, therefore, had many supporters.

Another theory maintains that bacteria have a typical nucleus and are in no way structurally different from ordinary cells. This opinion was suggested by Arthur Meyer, who claims to have succeeded in differentiating, in a great many bacteria, granules which fix nuclear stains, and of which one or often several appear in a cell. These granules he would consider nuclei. It seems to be established, however, that the majority of the elements noted by Meyer are not nuclei, but reserve products common among the *Protista* and known as metachromatic corpuscles.

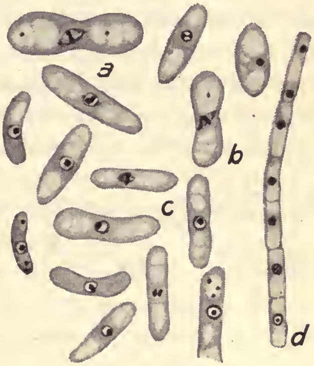

FIG. 70.—*Bacterium gammari* and a filamentous bacterium from the intestine of *Bryodrilus.* (*After Véjdowsky.*)

Véjdowsky's efforts have resulted in much weightier proofs in favor of the existence of a true nucleus. In the *Bacterium gammari*, a species discovered by him in the sections of a little fresh water crustacean, *Gammarus zschokkei*, Véjdowsky has been able to demonstrate in each cell a typical nucleus which is always present. This nucleus appears very clearly; it consists of a colorless nucleoplasm surrounded by a membrane and containing karyosomes (Fig. 70). The author had the good fortune to ascertain in several cases karyokinetic representations of the division of this nucleus (*a*, *b*, *c*). In short, the presence of this nucleus is indisputable.

The same author discovered a similar structure in a filamentous bacterium found in the digestive tract of an *Annelida* (*Bryodrilus ehlersi*) (Fig. 70, *d*).

These conclusions are positive, but the species observed by Véjdowsky are not well-defined bacteria, and may be thought to belong to the molds rather than to the bacteria. It has also been said, not without reason, that *Bact. gammari* might be a yeast of the genus *Schizosacchromyces* and that the filamentous bacterium studied by Véjdowski seems to resemble a filamentous mold.

However this may be, one of Véjdowsky's pupils, Mencl, has endeavored to apply these conclusions to other bacteria, which are well-defined, notably *B. megatherium*, but has only succeeded in bringing forth proofs which are much less convincing of the existence of a nucleus. The author strived to discover a nucleus, but this organ is not constant and does not show the structure of a true nucleus.

Both Kruis and Rayman have discovered a nucleus in different bacteria (*B. mycoïdes*, *radicosus*, etc.). This nucleus appears only in very young cells; it is not found in older cells, and seems (like the nucleus noted by Mencl) to represent merely the incipient transverse septum which fixes stains well at the beginning of its formation and in some ways resembles a nucleus.

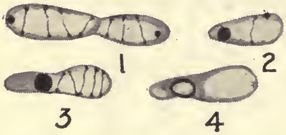

FIG. 71.—*Bacillus megatherium.* (*After Penau.*)

The studies of Penau, who also endeavored to prove the existence of a typical nucleus in bacteria, were no more successful. In *B. megatherium*, he describes the following phases. In the youngest cells he observes a stage where the cytoplasm is very dense and uniformly stained, without a trace of differentiation. Immediately succeeding is a phase where the cytoplasm becomes less chromatic and is filled with vacuoles. At this point the author finds in each cell a tiny granule (Fig. 71, 1), homogeneous and easily stained, situated at one of the poles of the cell, very near the membrane. This granule he considers to be a nucleus. Moreover, in the cytoplasmic web he observes a series of stainable granules connected by slender trabeculæ, thus forming a kind of network which he likens to mitochondrial and chromidial formations. At the time of sporulation, Penau finds an increase in the size of the nucleus (Fig. 71, 2 and 3) which changes to a large granule; this is soon surrounded by a membrane and becomes the spore (4), which is therefore formed mostly of chromatin.

The same author discovers a very different structure in *Bact. anthracis*. Here, after a stage of undifferentiated structure which characterizes the youngest cells, follows a phase where the cytoplasm becomes alveolar. At this time, at one of the poles of each cell, appears a very large homogeneous granule which Penau regards as a nucleus. This nucleus, however, has only an ephemeral existence and quickly undergoes a cytolysis during which it disintegrates. The disintegration products then impregnate the trabeculæ of the cytoplasm and the nucleus becomes diffuse. In a last phase which corresponds to sporogenesis, the chromatin which impregnates the cytoplasm is partly condensed at one of the poles, where it forms first a mass of grains, then a large granule which changes to a spore.

Nothing is less conclusive than these results, since the author cannot discover an homologous structure in the different species which he studies, and since the nucleus which he describes is only a transitory organ not showing the distinguishing characteristics of a nucleus.

To prove the existence of a nucleus in bacteria, it is necessary to show a nucleus with a differentiated structure, the constant presence of the nucleus, and to follow the division of this organ during the cellular separation. So far no one has apparently been able to differentiate such an organ in well-defined bacteria. We must conclude, therefore, that with the exception of the results obtained by Véjdowsky, all observations so far gathered in favor of the existence of a typical nucleus in bacteria are by no means convincing.

The third theory asserts the existence of a *diffuse nucleus* in bacteria. It was first suggested by Weigert and more carefully formulated by Bütschli. This author describes in a certain number of *Sulpho-bacteria* of large size, *Beggiatoa*, *Chromatium*, a kind of *central body* occupying nearly the whole volume of the cell and consisting of an alveolar cytoplasm of highly stainable web, containing within its knots numerous chromatic granulations (Fig. 72). The remainder of the cell consists

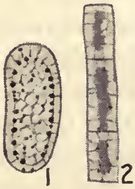

FIG. 72.—1. *Chromatium okenii*. 2. *Beggiatoa alba*. These two bacteria have a central body containing chromatic grains and considered by Bütschli as the equivalent of a nucleus. (*After Bütschli.*)

of a thin cytoplasmic layer, less easily stainable, surrounding the central body. Bütschli compares this structure with the one which has been demonstrated in the *Cyanophyceæ*, and claims that the central body represents the equivalent of a nucleus. It would be a sort of large nucleus occupying most of the cell, not bounded by a membrane, and scarcely distinct from the cytoplasm. This structure has recently been verified in *Chromatium okenii* by Dangeard. The *Sulpho-bacteria*, however, are organisms morphologically entirely distinct from ordinary bacteria, and are apparently directly related to the *Cyanophyceæ*. Such a structure is not found in other bacteria, in which it is impossible to demonstrate a central body and in which, one must admit, the nucleus is still more diffuse.

To Schaudinn we are indebted for the most exact observations in favor of the theory of the diffuse nucleus. He had the good fortune to discover in the intestine of the cockroach, *Periplaneta orientalis*, a bacillus of very large size which he named *B. bütschlii*. It is the largest bacillus known at present (4μ wide), and lends itself readily, therefore, to cytological studies. His minute observations have shown that there is no nucleus, the cells enclosing a finely alveolar cytoplasm, whose net contains many small grains which take nuclear stains (Fig. 73, 1–6).

At the time of sporulation the chromatic grains increase in size (Fig. 73, 7–9), then gather at the center of the cell in a kind of axial wreath (Fig. 73, 10). The two extremities of this wreath quickly swell with an accumulation of chromatic grains and form two granular masses, one at either pole. These two masses form the beginning of the two spores, for each cell forms two spores (Fig. 73, 11 and 12). The grains which compose these two rudiments then condense to form two large homogeneous granules (Fig. 73, 13) which strongly resemble nuclei and which Schaudinn considers to be such. Around these two granules is soon condensed a thin cytoplasmic zone which in turn is separated from the surrounding cytoplasm by a membrane (Fig. 73, 13). Henceforth the spores cannot be stained by ordinary means because of the thickness of their membrane which prevents the penetration of stains (Fig. 73, 14). The granules of the wreath, which join the two rudiments of spores, gradually disappear as well as the cytoplasm, while the spores increase in size. Then the sporangium ends by breaking and setting free the two spores. Germination consists simply of a swelling of the spore, then the formation of a small rod which issues from the spore and forms a septum for itself (Fig. 73, 15 and 16). As soon as the spore germinates, the nucleus ceases to exist as a morphologic entity; it is scattered in the cytoplasm in the form of little grains.

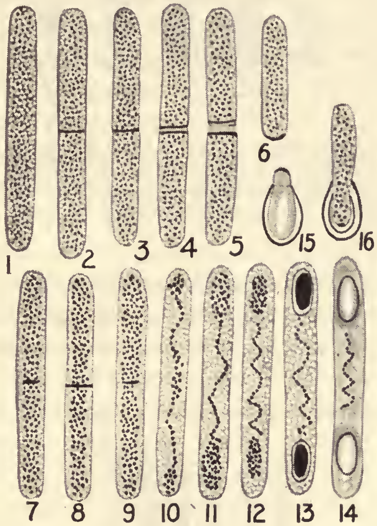

FIG. 73.—*Bacillus bütschlii.* 1–16, Vegetative cells and their division. 7–9, Beginning of sporulation: the cells about to sporulate are partitioned off crosswise; then the septum thus formed is absorbed, at which time sporulation begins. Schaudinn considers this partitioning off followed by fusion of the two daughter cells as a rudimentary sexuality. 10–13, Formation of the beginnings of the two spores, at the poles of the cell. 14, Ripe spores. 15–16, Germination of the spore. (*After Schaudinn.*)

In another bacillus smaller in size (*B. sporonema*), Schaudinn has found an analogous structure only at the time of sporulation; he does not prove the formation of an axial filament but only the condensation of a portion of the chromatic grains into a large granule which forms the beginning of the spore (Fig. 74).

By the fact that in these two bacilli the beginning of the spores appears as a granule equivalent in some respects to a nucleus and resulting from the condensation of a portion of the stainable grains, Schaudinn is led to believe that these grains are composed of chromatin and represent a kind of diffuse nucleus.

These results have been confirmed by our studies of a large number of endospore bacilli (*B. megatherium, radicosus, mycoides, asterosporus, alvei*). Upon examination at the very outset of their development, these bacteria present a homogeneous appearance and are uniformly stained with no great differentiation, explicable by the density of the cytoplasm or by a special condition of the membrane. At this stage the cells are in the process of active divisions, after which the transverse septa are formed as follows: On the side walls of the bacillus appear two small granules which take some stains (Fig. 75, 1). These soon disintegrate at the center of the cell to form a thin band marking out the two daughter cells and forming the beginning of the transverse septum. This strongly resembles a nucleus and has apparently been considered as such by a number of authors (Rayman and Krius, Mencl).

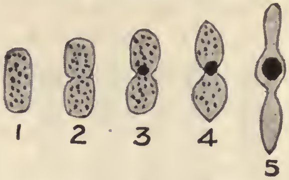

FIG. 74.—*Bacillus sporonema.* 1, Cell about to sporulate. 2, This cell grows narrow at the center, as if it were going to be divided (Schaudinn regards this pinching together which afterward disappears (5), as the vestige of an ancestral sexuality like that of *B. bütschlii*). 3–5, Formation of the beginning of the spore. (*After Schaudinn.*)

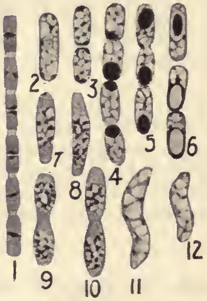

FIG. 75.—1–10, *Bacillus radicosus.* 1, Beginning of development. 2–3, Cells at the end of eight hours; 4–6, sporulation. 9–10, Cells in which the chromatic grains are located in the middle in a mass slightly resembling a nucleus. 11–12, *Spirillum volutans.*

Toward the eighth hour of development, the cells show clearly their structure which is changed in appearance; the cytoplasm vacuolizes and ends by displaying a fine alveolar structure. The web contains in its knots small, highly stainable granules (Fig. 75, 2 and 3). In some cases (cultures on special media for example), there is noticeable a localization of these granules at the center of each cell, forming a granular region which recalls somewhat the appearance of a large nucleus and which is separated into two portions at the time of the cellular division as if it were indeed a true nucleus (Fig. 75, 7 and 10).

These granules fix the nuclear stains, and it seems permissible to consider them chromatic in nature.

At the time of sporulation there forms at one of the poles of the cell a small oval mass, easily stained, which is like a nucleus in appearance (Fig. 75, 4 and 5). This results from the condensation of part of the chromatic granules of the cytoplasm, gradually grows larger, and changes to a spore. When the spore has reached a certain size, it is surrounded by a membrane which prevents the penetration of ordinary stains (Fig. 75, 6); it appears then like a large colorless sphere in the stained cytoplasm of the cell (Fig. 75, 6).

At no stage of the development have we observed the least trace of a nucleus. May there be a nucleus which our present technic would not enable us to differentiate? That has seemed to us scarcely probable, for if this nucleus existed, it would certainly be visible in a species as large as *B. bütschlii* and would not have escaped Schaudinn. The most reasonable hypothesis, the one which we have adopted, is to consider like Schaudinn that bacteria contain chromatin more or less mingled with cytoplasm, differentiated in the case of small grains and condensing at the time of sporulation to form the spore which would *consist principally of chromatin.* The cells of bacteria would accordingly have a very primitive structure.

Granted the clearly demonstrated existence of this particular structure in the *Cyanophyceæ*, there is no reason for not admitting that the nucleus, very rudimentary in the *Cyanophyceæ*, might be even more so in bacteria, being reduced to a diffuse nucleus consisting of chromatic grains scattered in the cytoplasm.

These observations have, moreover, received a series of new confirmations by the labors of a great many authors (Swellengrebel, Ruzicka, Ambrez, etc.) and especially by the later researches of Dobell. The latter investigator discovered, in the intestines of frogs and toads, a large bacillus (2μ wide) almost as large as *B. bütschlii*, and named it, *B. flexilis*. This species shows exactly the same cytological characteristics as *B. bütschlii* (Fig. 76).

Through a study of a number of different bacteria found in the intestine of toads, frogs and lizards, Dobell has endeavored to show that this diffuse nucleus is not original, but derived from the retrogression of a more highly differentiated nucleus.

Thus in various micrococci he was able to show in each cell the existence of a central stainable granule, dividing by constriction at the time of cellular division, and which he regards as a nucleus (Fig. 77, 1–5). In other cocco-bacillary species of bacteria characterized by spherical shape capable of elongation, Dobell discovers a similar nucleus in the spherical cells. When the cell lengthens and assumes the appearance of a bacillus, this nucleus changes to a spiral axial filament (Fig. 77, 5 and 6).

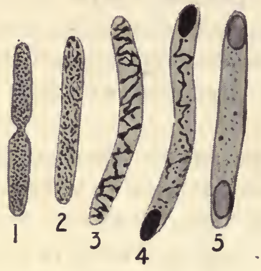

FIG. 76.

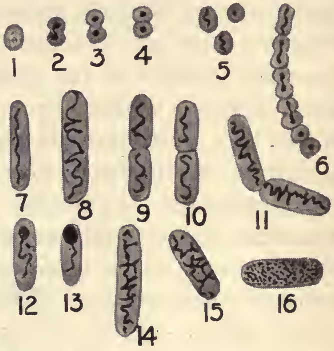

FIG. 77.

FIG. 76.—*Bacillus flexilis.* 1, Beginning of the division of a cell about to sporulate (vestige of sexuality). 2, Disappearance of the incipient division. 3, Formation of the chromatic axial filament. 4, Formation of the beginning of two spores. 5, Ripe spores. (*After Dobell.*)

FIG. 77.—Various bacteria, showing the successive types of the retrogression of the original nucleus and its transformation to a diffuse nucleus. (*After Dobell.*)

In various bacilli the same author demonstrates a filament which is ever present (Fig. 77, 7–11). The spore results from the condensation, at one of the poles, in the shape of a large chromatic granule, of part of the grains which compose this filament (Fig. 77, 12 and 13). An interesting variation of this structure is found in *B. saccobrinchi.*

In this bacillus is noticed first an initial stage where the nucleus is represented by an axial filament quite similar to that of *B. spirogyra* (Fig. 77, 14). In the course of development, however, this filament resolves itself into a great many grains which scatter through the cell (Fig. 77, 15 and 16). The nucleus then becomes diffuse. Part of this diffuse nucleus next condenses at the time of sporulation into a large chromatic grain which forms the beginning of the spore. Finally, in other bacilli, Dobell finds in the whole development no more than a diffuse nucleus, that is, the structure described by Schaudinn and by Guilliermond.

In the group of spirilla, Dobell notices these three types of structure: In some species he finds present a spherical body resembling a nucleus; other species show a zigzag or a spiral filament; still others have a diffuse nucleus.

From these observations, Dobell feels authorized to conclude that bacteria are organisms originally containing a nucleus, but in which the nucleus, as a result of parasitism, has undergone a series of retrogressions which have ended by making it diffuse.

This opinion would have the advantage of reconciling opposed theories. It would explain how some authors have been able to discern a true nucleus in various forms.

Another more weighty reasoning which might also explain these contradictions is the fact that under the name of bacteria are gathered forms perhaps very different, some of which seem to belong to the *Sulpho-bacteria* and others might be considered as molds.

Although we have just mentioned numerous works, the conclusion, to my mind, would be that while some bacteria may contain a more or less rudimentary nucleus whose existence is nowhere else precisely demonstrated, so far, in the great majority of the species, nothing more has been found than a diffuse nucleus consisting only of grains of chromatin scattered through the cytoplasm.

*Life Cycle of Bacteria**.—The life-cycle of bacteria will prove a very important factor in the study of their morphology, their cultivation, their cultural characteristics and their classification, if its development takes place along the line so definitely advanced by Löhnis and Smith†. The variation in the appearance of a species of bacteria has long been recognized; cultivation has been fraught with difficulties which have at times been in some way associated with the change in form or in a sense connected with "involution" alterations; cultural characteristics have likewise been subject to variations which have depended upon the so-called vigor of the organism; and classification of bacteria may be materially affected since some of the cycles approach closely those of protozoa.

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* Prepared by the Editor.

† Löhnis, F. and Smith, N. R.: Jour. Agr. Research, VI, 18, 675. 1916.

Perhaps the most significant changes upon which the life-cycle of bacteria is based may be those represented by Jones,[*] and Löhnis and Smith in the life of *Azotobacter-types*. The polymorphous character of the *Azotobacter* group has been a matter of intense interest for a long period. Löhnis and Smith have not only endeavored to follow the variations through a consistent historical developmental cycle but have attempted to organize their observations and have them in accord with past observations.

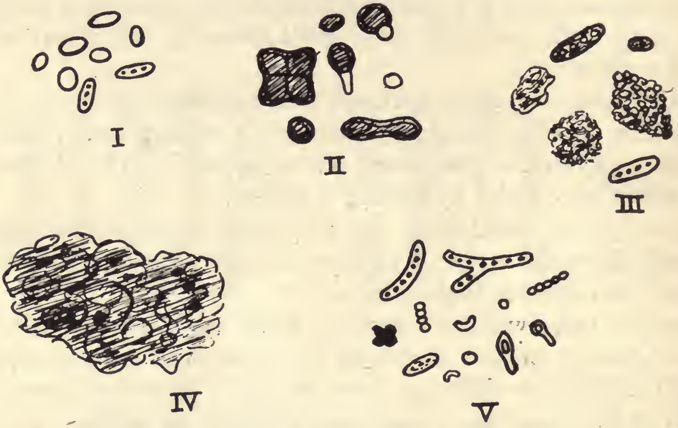

FIG. 78.—Change of *Azotobacter* from the normal cells (I) to arthrospores (II) and involution forms (III) to be lost in symplastic stage (IV) and recovering cell-form in V. Diagrammatic from *Löhnis and Smith.*

The organism may be assumed to exist in the form of a distinct cell and at other times in an amorphous condition called by the authors, the *symplastic stage*. In the usual cell-form the organism may multiply by fission as is the case with all bacteria, may produce endospores

[*] Jones, D. H.: Cent. f. Bact.; Trans Royal Society of Canada, 1913.

as is a common mode of reproduction, or arthrospores, when the entire organism appears to transmute to a resting stage or spore, or, the organism may pass to the amorphous or symplastic condition. There is also a possibility of a union or "conjunction" of cells suggesting the functioning of gametocytes.

In passing into the symplastic stage the cells passing through involution forms appear to form clumps and lose completely their individuality of form and contents in a general mass of disorganized protoplasmic debris. Presumably scattered throughout this mass exists what may be recognized in protozoal forms, yeast cells, et cetera, nuclear centers, for out of this more or less homogeneous unvarying background of protoplasmic substance appear many lines resulting in modified forms which pass on to forms similar to the original cellular forms from which this amorphous mass was at first derived.

The form of *Azotobacter* upon which this life-cycle theory is based may not be, of course, conclusive; however, Jones has confirmed many of the findings of Löhnis and Smith in the case of *Azotobacter* but is not ready to subscribe to all of their interpretations. Jones* claims, too, that so far as other species of bacteria are concerned in this theory of life-cycle, he has been unable to confirm Löhnis and Smith who assert that in the forty-eight species studied, they find practically the same developmental cycle.

This subject is of so wide importance that it deserves much attention and study.

Reserve Products.†—Besides the grains of chromatin which we have just been considering in bacteria are found other granulations which do not show the characteristics of chromatin and which act as products of nutrition. These granulations are characterized by the reddish color which they assume with most of the aniline blue or violet dyes, as well as with hæmatoxylin. These bodies, which are common to the majority of the *Protista*, are metachromatic corpuscles.

They are found in larger or smaller numbers according to the species, the age of the cells, and the medium in which they are living. Some bacteria contain few metachromatic corpuscles (*B. radicosus, megatherium, mycoides*); others produce many (*B. alvei, asterosporus, Sp. volutans, Bact. tuberculosis* and *diphtheriæ*). The metachromatic corpuscles appear at the beginning of development in the form of very small grains, which generally increase gradually in size during development, and finally are absorbed in the very old cells. They are sometimes distributed through the whole cell (*Spirillum volutans*) as grains of chromatin (Fig. 79, 8 and 9), but most often they tend to gather at the two poles of the cell, or line up all along the bacillus (Fig. 79, 1 to 4, 6, 10, 11). In some species (*B. alvei*, *asterosporus*, *Bact. tuberculosis* and *diphtheriæ*), these corpuscles grow bigger until they attain relatively large dimensions, surpassing the bacillus in size. Thus they cause a series of swellings all along the bacillus, which in consequence appears somewhat like a necklace (Fig. 79, 11). They then give the illusion of spores; one can easily understand the error of some authors who have confused them with spores, notably in the case of the *Bact. tuberculosis*.

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*Jones, D. H.: Jour. of Bact., Vol. V, p. 325.

†Prepared by A. Guilliermond.

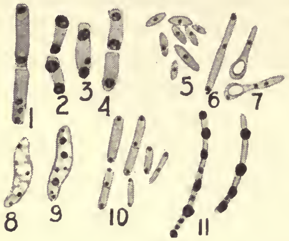

FIG. 79.—Various bacteria stained by a method which differentiates only the metachromatic corpuscles. 1–4, *Bacillus radicosus*. 5–6, *Bacillus asterosporus*. 7, The same. The cells have formed their spore and the metachromatic corpuscles outside of the spores have not yet been absorbed by it. 8–9, *Spirillum volutans*. 10–11, *Bacillus alvei*.

In *B. asterosporus*, the metachromatic corpuscles usually appear in the youngest cells, singly and in the shape of a small central granule closely resembling a nucleus and which A. Meyer seems to have taken for such (Fig. 79, 5).

During sporulation, the metachromatic corpuscles exist just outside of the spore (Fig. 79, 7), then are finally absorbed by it. They therefore act like reserve products.

Moreover, in the cells of bacteria other reserve products, notably globules of fat and of glycogen, have been found.

BACTERIAL CELL WALL.—*General Structure.**—All the bacteria have cell walls and it is these that give definite form to the cell. These walls are rigid and elastic and are probably made up of two layers, the outer one of which is able to deliquesce and form capsules, or perhaps zooglœa. The inner part retains the elasticity and gives the form to the bacteria. These cell walls are readily permeable to water and it is through them that all of the nourishment of the cell is obtained; that is, there are no openings for the entrance of food or the discharge of by-products, but the intake and output goes on through the cell wall which is entire.

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* Prepared by W. D. Frost.

*Minute Structure of Cell Wall.**—In some species of large size, the membrane can be distinguished when strongly magnified, and appears with a double contour. Usually it is scarcely visible, and can be observed only when the contents of the cell has been contracted by plasmolysis or by a suitable reagent. It is sometimes thin, sometimes more or less thick. In the latter case, it is often possible to recognize two layers, an *inner or cuticular layer*, very thin and transparent; and the other external, not so well defined and thicker, jelly-like in appearance. This latter or *gelatinous layer* seems to result from a special differentiation of the peripheral zones of the inner layer. The outer layer ordinarily resists staining reagents and appears as a kind of transparent zone about the colored elements. It can acquire a relatively great thickness, and the formations described as *capsules* are only an exaggeration of this gelatinous layer.

Schaudinn has been able to observe quite carefully the construction of the cuticular layer in *B. bütschlii.* According to him, the membrane seen in profile would appear to consist of a series of disks alternately clear and cloudy (Fig. 80, *A* and *B*). Seen from the front, it would give the impression of a network whose meshes are more refringent and stain more highly (*C*). It is laid on a peripheral zone of cytoplasm, a kind of ectoplasm with closer network, and is clearly differentiated from the rest of the cytoplasm. The spore is provided with a double membrane and has at one of its poles a sort of micropyle through which germination is effected (Fig. 73, 15 and 16).

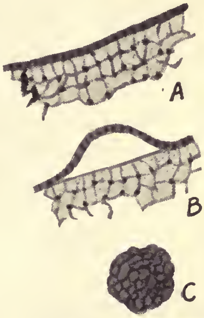

FIG. 80.—*A* and *B*, Structure of the membrane and of the ectoderm in *Bacillus bütschlii.* *C*, Membrane of the same bacillus, front view. (*After Schaudinn.*)

The chemical composition of the membrane is little known. According to some authors, this membrane consists of cellulose; according to others, it contains a lipoid substance; finally, by many authors it is supposed to be composed principally of nitrogenous compounds. Let us remark further that chitin has supposedly been detected therein.

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* Prepared by A. Guilliermond.

*Capsules.**—A considerable number of the bacteria regularly, or under certain conditions, form what are known as capsules (Fig. 81). These are mucilaginous envelopes which in width frequently exceed that of the organism itself. In microscopical preparations of bacteria it is important to differentiate these from artifacts, since by ordinary staining methods the capsules are not colored but appear as colorless areas surrounding the bacteria. If, due to shrinkage of the bacteria, or other material on the preparation, clear spaces are formed, it is readily seen that these might be confused with the real capsule. It is possible to stain the capsules by special methods; these must be used in order to determine positively the existence of the capsules. The bacteria which grow in the bodies of animals frequently contain these capsules but fail to show them when grown upon artificial culture media. It is difficult, therefore, to determine whether or not an organism has a capsule by mere examination of cultures. Some culture media, however, do cause a formation of capsules in the case of capsulated bacteria. These are blood serum, sometimes, and milk, usually. Beautiful capsules can be obtained by growing such bacteria as the *Bact. pneumoniæ*, *Bact. capsulatum*, and *Bact. Welchii* in milk cultures. *Strept. mesenteroides* is a bacterium which grows in the syrup of the sugar refineries and forms abundant capsules. This organism changes the char-

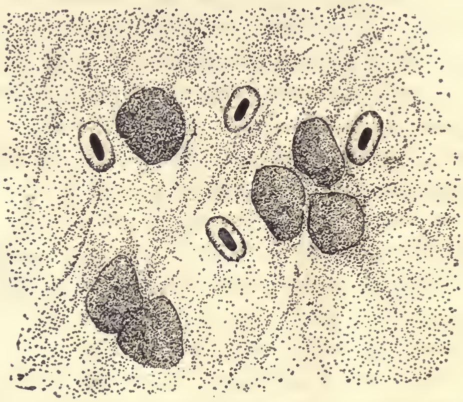

FIG. 81.—Capsules. *Bact. pneumoniæ* (Friedlander). (*After Weichselbaum from Frost and McCampbell.*)

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* Prepared by W. D. Frost.

acter of the syrup, and its entrance and growth is frequently the cause of serious loss.

FLAGELLA.—*General Consideration of Flagella.**—The flagella are very narrow thread-like structures. It is not known how narrow since they cannot usually be seen without staining and they can only be stained by precipitating some chemical which may add considerably to their width. They are frequently longer than the organism which possesses them and sometimes many times that length. *B. symptomatici anthracis* found in the soil has a flagellum sixty times its own length. The arrangement of the flagella on the bacteria is quite constant

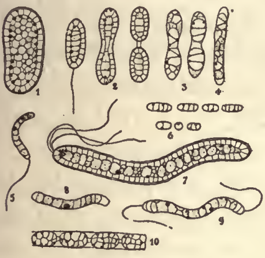

FIG. 82.

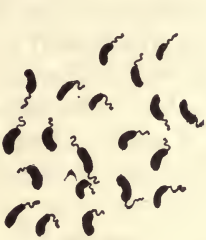

FIG. 83.

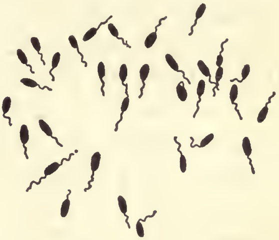

FIG. 84.

FIG. 82.—*Chromatium okenii;* 2, *Bacterium lineola;* 3, 4 and 5, sulpho-bacteria; 7, *Ophidomonas jenensis;* 8, and 9, *Spirillum undula;* 10, *Cladothrix dichotoma.* (*After Bütschli from Guilliermond review, Bull. Inst. Past.*)

FIG. 83.—*Microspira comma.* Monotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

FIG. 84.—*Pseudomonas pyocyanea.* Monotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

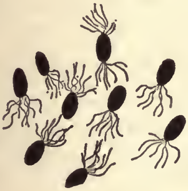

FIG. 85.

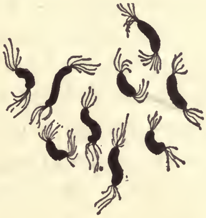

FIG. 86.

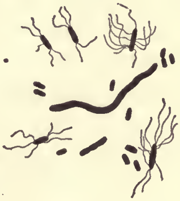

FIG. 87.

FIG. 85.—*Pseudomonas syncyanea.* Lophotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

FIG. 86.—*Spirillum rubrum.* Lophotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

FIG. 87.—*Bacillus typhosus.* Peritrichous bacteria. (*After Migula from Schmidt and Weiss, and Frost and McCampbell.*)

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* Prepared by W. D. Frost.

and is used by some authors to differentiate genera. Very few of the micrococci are provided with flagella, as was indicated above, and in the bacilli and spirilla they may be arranged at the poles singly or in brushes, or they may be arranged on the entire periphery of the cells. When bacteria are provided with a single flagellum at one pole, the arrangement is said to be *monotrichous* (Figs. 82, 83 and 84). When they are arranged in brushes, the arrangement is spoken of as *lophotrichous* (Figs. 85 and 86) and when they are arranged on the entire periphery, the arrangement is said to be *peritrichous* (Fig. 87). It frequently happens that in the case of the monotrichous and lophotrichous the flagella occur at both ends of the organism. This is explained by the fact that the organism is just undergoing binary fission and that the second group is on the newly forming cell. It is worth while in this connection to call attention to the fact that the flagella on one end are new, while those on the other end may be thousands of generations old.

*Minute Consideration of Flagella.**—The question of the cilia or flagella of bacteria is not yet entirely decided. The absence of cilia in large bacteria capable of motion gives the idea that these are not the only organs of motion, and that contraction of the protoplasm certainly plays the most important rôle in the phenomena of motility. Moreover, the nature of cilia has been debated. Van Tieghem and Bütschli, taking their stand primarily on the difficulty of staining cilia by the reagents which rapidly color protoplasm, have considered these cilia to be simply prolongations of the membrane, lacking all contractibility and locomotive power. According to Van Tieghem, when two cells formed by the division of the same element separate, the common portion of the transverse septum, instead of dividing neatly in two, can stretch out into a filament which breaks at a greater or less distance from each of the two daughter cells. This prolongation composes the vibratile cilium.

This theory, however, does not explain the existence in certain bacteria of clusters of cilia at the two poles, or of cilia distributed over the whole surface of the membrane. Other authors, as for example A. Fischer, consider the cilia true prolongations of the protoplasm issuing through tiny apertures in the membrane. This view at present tends more and more to predominate, and the existence of flagella on bacteria appears to be demonstrated.

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* Prepared by A. Guilliermond.

Another interesting peculiarity, moreover, has recently been established independently by Swellengrebel and by Dangeard. According to these authorities, in some species (*Chromatium okenii* and *Spirillum volutans*) the cilia have connection with one of the chromatic grains of the diffuse nucleus. There is a chromatic filament starting from the base of the cilium and ending in connection with a chromatic grain, similar to the organisms with flagella in which the flagellum is in relation to a basal chromatic grain (blepharoplast).

THE HIGHER BACTERIA*

The so-called higher bacteria include some of the spiral forms, at least the larger spirochætes, the thread or *trichobacteria*, and the sulphur or *thiobacteria*.

The spirochætes and *trichobacteria* contain so many forms of interest that their form and structure needs special consideration.

THE LARGER SPIROCHÆTES.—Spirochætes differ so much among themselves that it seems necessary to divide them into two groups. The members of one of these groups, the small spirochætes, are practically identical with the true bacteria, and naturally fall in the family of the *Spirilliaceæ*. Members of this group, however, so gradually approach the other group, the large spirochætes, that it is difficult to draw a line of separation between the two, yet the large spirochætes resemble in so many essential details the trypanosomes that they are usually placed as a coördinate genus with them under the flagellates—a sub-class of the *Protozoa*. The larger spirochætes are described as follows:

Form and Size.—In form the spirochætes are long, very thin and flexible spirals. Their length is usually not less than twenty times their breadth. Some forms are as long as 500 μ. It seems probable that some of them are flattened and hence in form are more like a spirally bent ribbon than rod.

Motility.—These organisms move very rapidly under normal conditions. The character of the movement may be of three kinds: (1) Lashing, eel or snake like; (2) undulatory, compared to the flapping of a sail in the wind; (3) rotation, similar to a cork-screw when pushed into a cork.

Reproduction.—Multiplication is by means of binary fission. If these forms are to be considered as bacteria, the division would be expected to be by means of transverse partition walls. A number of

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* Prepared by W. D. Frost.

workers, however, have described a process of longitudinal division. Forked forms also which are frequently seen are held to indicate longitudinal divisions. Some observers have claimed that conjugation occurs among the spirochætes. If this is true their relation to the *Protozoa* would be quite likely, but accounts of this phenomenon are inconclusive. Several observers have described "rolled up" specimens, oval and ovoid forms, which have been assumed to be cysts. The spirochætes break up into granules or short segments and such specimens are sometimes spoken of as "monili form." It is not definitely known whether these coccoid forms are simply degenerative forms or the equivalent of bacterial spores.

Sheaths.—A definite sheath has been described for some forms and the irregularity in the disposition of this around the cell may account for the structures that have been taken for undulating membranes.

Cell Aggregates.—There is apparently no definite cell grouping but tangled masses of these organisms have been described in several species.

THE TRICHOBACTERIA.—The *trichobacteria* (*Chlamydobacteriaceæ*) are thread or filamentous forms. The cells are cylindrical and similar in form and may or may not vary in size in different parts of the filament. The individual cells are capable of independent existence, but when growing in the filament give evidence of differentiation in function. Sometimes these filaments are attached to the substratum or some object in it; at other times they are free. In case of the sessile forms the cells at the attached end (base) are smaller than those at the apex. In other members of the group the ends of the thread are swollen or become club-shaped (Fig. 88). In some forms cell division takes place in three directions of space, thus forming a thread of massed cells.

Branching.—The filaments are usually unbranched, but some forms show true branching, such as is found among the plants—fungi and algæ. Some again exhibit what is called false branching. This is due to a misplaced cell, which grows parallel or at an angle to the parent thread and suggests branching.

Reproduction.—The cells throughout the filament may divide to form spores, but the apical cells of the thread are frequently set apart for the purpose of reproduction, and by a process of division form spores or conidia. The conidia are usually round and without any resting stage may produce new threads of cells. Sometimes spores germinate while still in the old thread (Fig. 88), giving a tangled mass of cells or whorls of new threads at intervals on the old. The conidia may be either motile or non-motile. The motility of these conidia when it exists is due to flagella.

Sheath.—The threads of cells are sometimes surrounded by sheaths of varying thickness. This sheath is a thickened and hardened membrane, and forms a tube in which the different cells of the bacteria are contained. This sheath is homologous to a capsule. In it are frequently deposited characteristic by-products of the cell. In *Crenothrix* (an iron bacterium), for example, we have iron oxides.

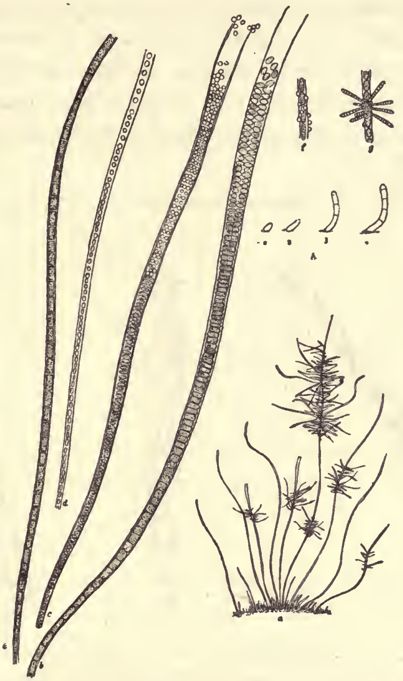

FIG. 88.—*Crenothrix polyspora* Cohn, Brunnenfaden. (*After Migula from Schmidt and Weiss.*)

Among the iron bacteria are several interesting forms. *Crenothrix polyspora* is one of the best known. Its general morphology is shown in Fig. 88. The attached, sessile, threads are shown at *a*. The tufts of short threads, radiating from the larger threads, are formed by the germination of conidia while they are still in the parent threads. The large threads, *b*, *c*, *d*, and *e*, show more details. In *e* a uniform thread is shown with the separate vegetative cells; in *d* these have broken up into conidia. The flaring form of the threads are shown in *c* and *b* where the conidia are formed in large numbers. These figures also show the sheath which is indicated by the double line in *c* and by the extension of the lines beyond the cell contents.

Chlamydothrix ochracea Migula is composed of filamentous, cylindrical, colorless threads. The sheath is at first thin and colorless but later becomes thicker, yellow or brown due to encrustations of iron oxide. Multiplication is by means of cell division and swarm cells. These latter may sometimes germinate in the sheath, giving the appearance of branching (Fig. 89, *c*).

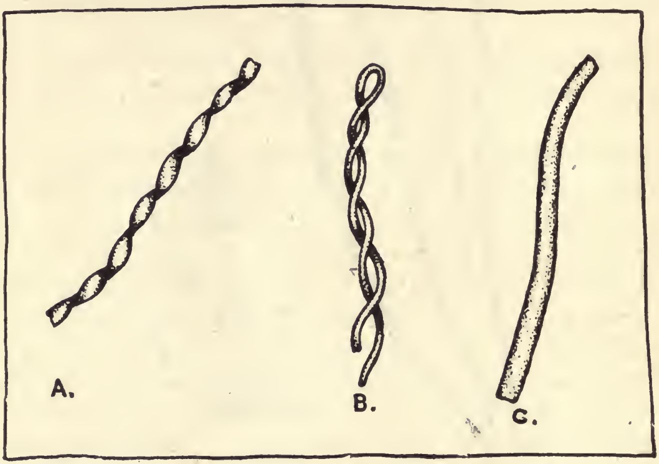

FIG. 89.—*A*, *Spirophyllum ferrugineum;* *B*, *Gallionella ferruginea;* *C*, *Leptothrix ochracea.* × about 1080. (*After Harder.*)

Gallionella ferruginea Ehr., in its typical form, consists of spiral threads coiled together in double or quadruple coils like a rope. The threads are cylindrical but comparatively thin. Individual cells have not been distinguished in the threads (Fig. 89, *B*).

Spirophyllum ferrugineum Ellis is very similar to and associated with the above. It differs principally in the shape of the threads which are flat or ribbon-like. The threads are always twisted but may occur singly or be coiled into ropes (Fig. 89, *A*).

All of these iron bacteria have the power of changing certain soluble salts of iron into insoluble forms and thus precipitate them from solution. Growing in the pipes of a city water supply their deposits choke up the pipes and hence they are frequently referred to as "water pests." As a result of researches in recent years these iron bacteria are now regarded as important geological agents and to them is ascribed a large share in the deposition of iron ores.

Other thread bacteria of considerable importance are the *actinomycetaceæ.* Some of them are common in the soil and recently have been given special study. Others cause disease and a well known form, *Actinomyces bovis* Hartz, is the cause of lumpy jaw in cattle.

The actinomycetes are mold-like organisms and often show true branching. They reproduce vegetatively or by means of conidia. They are without sulphur granules, not colored with bacteriopurpurin and the sheaths, if present, are not impregnated with iron. The structure of *Actinomyces bovis* is shown in Fig. 165, p. 780, while the characteristic radiating clubbed ends of the filaments, as these organisms grow in the tissues of cattle, are shown in Fig. 164, p. 779.

THE SULPHUR BACTERIA.—The sulphur bacteria are filamentous forms which may reach a length of many microns. They are cylindrical or perhaps sometimes flat. They may be either attached or actively motile. The movement when present is due not to flagella, but to an undulatory motion like that of the spirochætes or *Oscillaria* among the algæ. As they move forward they rotate on their own axis and swing their free ends.

Spore formation is unknown in some forms where multiplication is accomplished by the breaking up of the threads in short segments. In the case of the sessile forms conidia are produced at the end of the thread and are motile (*Thiothrix nivea*). The sulphur bacteria contain at certain stages strongly refractile sulphur granules in their bodies.

CLASSIFICATION*

The classification of bacteria was early recognized by Mueller as a matter of difficulty, since he says: "The difficulties that beset the investigation of these microscopic animals are complex; the sure and definite determination (of species) requires so much time, so much of acumen of eye and judgment, so much of perseverance and patience, that there is hardly anything else so difficult."

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* Prepared by W. D. Frost.

A considerable number of systems for the classification of the bacteria have been proposed. One of the most widely used at the present time is that devised by Migula. His system is based on the principle, universally followed by botanists and zoölogists, of using morphological characters only to distinguish genera. There has been, however, a growing conviction among bacteriologists that it is necessary to take physiological characters into consideration in determining even the major groups of bacteria in any system of classification. This revolutionary doctrine was presented in an extreme form by Orla Jensen who used the metabolic processes of the bacteria as the chief criteria for establishing not only genera but families and orders as well. A Committee of the Society of American Bacteriologists have recently reported on the Families and Genera of Bacteria*. This system makes use of both morphological and physiological characters and promises to be an important step towards a natural system of classification. Migula's system and that of the Committee of the Society of American Bacteriologists, in skeleton form, follow:

MIGULA'S CLASSIFICATION

Orders of the Schizomycetes

Cells contain sulphur. Colorless or pigmented rose, violet or red by bacteriopurpurin—never green..THIOBACTERIA
Cells free from sulphur and bacteriopurpurin, colorless or faintly colored....................EUBACTERIA

Families of Eubacteria

Cells globose in a free state, not elongating in any direction before division into 1, 2 or 3 planes....COCCACEÆ
Cells cylindrical, longer or shorter, and only dividing in one plane, and elongating to twice the normal length before division................
1. Cells straight, rod-shaped, without sheath, non-motile or motile by means of flagella...BACTERIACEÆ
2. Cells crooked, without sheath.............SPIRILLACEÆ
3. Cells inclosed in a sheath...................CHLAMYDOBACTERIACEÆ

Genera of the Coccaceæ

Cells without organs of locomotion
1. Division in one plane....................Streptococcus
2. Division in two planes....................Micrococcus
3. Division in three planes....................Sarcina

Cells with organs of locomotion
1. Division in two planes....................Planococcus
2. Division in three planes....................Planosarcina

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* Jour. Bact. II, p. 505, 1917.

Genera of the Bacteriaceæ

Cells without organs of locomotion..............Bacterium
Cells with organs of locoomtion
1. Flagella distributed over the whole body....Bacillus
2. Flagella polar..............................Pseudomonas

Genera of the Spirillaceæ

Cells rigid not snakelike or flexuous
1. Cells without organs of locomotion..........Spirosoma
2. Cells with organs of locomotion
(a) With one, very rarely two or three polar flagella..........................Microspira
(b) Cells with polar flagella in tufts of five to twenty.........................Spirillum
Cells flexuous...............................Spirochæta

Genera of the Chlamydobacteriaceæ

Cell contents without granules of sulphur
1. Cell threads unbranched
(a) Cell division always only in one plane..Chlamydothrix
(b) Cell division in three planes previous to conidia formation
i. Cells surrounded by a very delicate, scarcely visible, sheath (marine).....................Phragmidiothrix
ii. Sheath clearly visible (in fresh water).......................Crenothrix
2. Cell threads branched (pseudobranches).....Sphærothrix

Families of the Thiobacteria

Filamentous bacteria which do not contain bacteriopurpurin. Cells contain sulphur granules..BEGGIATOACEÆ
Cells contain bacteriopurpurin, sulphur granules may also be included.........................RHODOBACTERIACEÆ

Genera of the Beggiatoaceæ

Cells non-motile, threads attached to some object..Thiothrix
Moves by means of an undulating membrane.....Beggiatoa

Genera of the Rhodobacteriaceæ

This family includes twelve genera as follows: Thiocystis, Thiocapsa, Thiosarcina, Lamprocystis, Thiopedia, Amœbobacter, Thiothece, Thiodictyon, Thiopolycoccus, Chromatium, Rhodochromatium and Thiospirillum.

THE FAMILIES AND GENERA OF THE BACTERIA

Report of the Committee of the Society of American Bacteriologists. C.-E. A. Winslow *et al.* (Artificial key)

ORDERS OF THE SCHIZOMYCETES

- Cells united during the vegetative stage into a pseudoplasmodium.............................MYXOBACTERIALES
- Cells not forming a pseudoplasmodium
  - Cells free or united in elongated filaments, often with a well defined sheath. Conidia frequently formed. Free sulphur, iron or bacteriopurpurin often present.
    - Cells typically containing granules of sulphur or bacteriopurpurin or both..................THIOBACTERIALES
    - Sulphur and bacteriopurpurin absent; iron often present.................................CHLAMYDOBACTERIALES
  - Cells never in sheathed filaments. Conidia only in mycelial Mycobacteriaceæ. Flagella often present. Free iron, sulphur, or bactiopurpurin never present.............................EUBACTERIALES

FAMILIES OF THE EUBACTERIALES

- Cells spiral with polar flagella... .. ..IV. SPIRILLACEÆ
- Not as above
  - Cells spherical; rarely, if ever, motile; spores never produced; never securing growth energy from nitrogen or ammonia...................V. COCCACEÆ
  - Not as above
    - Cells short rod-shaped with a single, rarely two, polar flagellum; usually forming green or yellow pigment.......... ....III. PSEUDOMONADACEÆ
    - Not wholly as above
      - Spores formed..............................VIII. BACILLACEÆ
      - Spores never formed
        - Metabolism simple, securing growth energy from carbon, hydrogen, or their simple compounds; flagella, if present, polar......I. NITROBACTERIACEÆ
        - Metabolism complex, dependent upon more complex carbohydrate and protein substances; flagella, if present, peritrichic. Cells clubbed, fusiform, filamentous, branching or mycelial; those not distinctly so are either acid-fast or show barred irregular staining......................II. MYCOBACTERIACEÆ
        - Not as above
          - Gram positive; non-motile..............VII. LACTOBACILLACEÆ
          - Gram negative; often motile.............VI. BACTERIACEÆ

GENERA OF THE EUBACTERIALES

I. NITROBACTERIACEÆ

- Fixing nitrogen or oxidizing its compounds
  - Fixing free nitrogen
    - Cells large; in soil....................7. Azotobacter
    - Rods minute; in roots of leguminous plants..........................8. Rhizobium
  - Oxidizing nitrogen compounds
    - Oxidizing ammonia..................5. Nitrosomonas
    - Oxidizing nitrites...................6. Nitrobacter
- Not as above
  - Oxidizing hydrogen...................1. Hydrogenomonas
  - Oxidizing carbon compounds
    - Oxidizing alcohol; branching forms common.........................4. Mycoderma
    - Not as above, using simpler carbon compounds
      - Oxidizing CO......................3. Carboxydomonas
      - Oxidizing CH_4....................2. Methanomonas

II. MYCOBACTERIACEÆ

- Slender rods staining with difficulty and acid fast..............................3. Mycobacterium
- Not as above
  - Mycelium and conidia formed
    - With aerial hyphæ and conidia; usually saprophytic soil organisms.........2. Nocardia
    - Hyphæ and conidia not aerial; usually parasitic in animals...............1. Actinomyces
  - Not as above; cells rod-like, usually somewhat curved, clubbed, fusiform, or even branched, but never mycelial
    - Thick, long threads, fragmenting into short thick rods..................6. Leptotrichia
    - Not as above
      - Cells usually elongate and fusiform, filaments, if formed not branching; stains somewhat irregularly..5. Fusiformis
      - Cells slightly curved, clubbed, or in old cultures even branching; not filamentous; showing definite barred staining....................4. Corynebacterium

III. PSEUDOMONADACEÆ

- Generic characters mainly those of family..1. Pseudomonas

IV. SPIRILLACEÆ

- Flagellum single (rarely 2 or 3)............1. Vibrio
- Flagella tufted (5 to 20)..................2. Spirillum

V. COCCACEÆ

- Abundant red-pigmented growth on agar..7. Rhodococcus
- Not as above
  - Gram negative
    - Normally in pairs of flattened cells; growth on plain agar scanty, never bright yellow......................1. Neisseria
    - Normally in plates, packets, or irregular masses; growth on plain agar abundant, pigment definitely yellow
      - Cells in regular packets............6. Sarcina
      - Cells not in regular packets........5. Micrococcus
  - Gram positive (exceptions rare and not easily confused with above genera)
    - Cells normally in chains, sometimes in pairs (especially in acid environment) never in large irregular masses. Gelatin rarely liquefied. Growth on plain agar usually translucent, never heavy, never yellow or orange......2. Streptococcus
    - Cells normally in groups and masses; (occasionally in plates in Albococcus) chains short and irregular, if present. Gelatin often liquefied. Agar growth abundant, white to orange.................
      - Pigment orange (rarely lacking); gelatin often liquefied actively....3. Staphylococcus
      - Whitish to porcelain white; liquefaction less vigorous...............4. Albococcus

VI. BACTERIACEÆ

- Plant pathogens........................2. Erwinia
- Not as above; saprophytes or in animal habitats (intestines, tissues, etc.)
  - Usually motile and exhibiting active fermentative powers; typically parasitic in intestines of man and higher animals; growing well on ordinary media.............................1. Bacterium

Not wholly as above
  Growing only in presence of hemoglobin, ascitic fluid or serum.........4. Hemophilus
  Growth on media scanty, but less sensitive than the above; short rods with tendency to bipolar stain......3. Pasteurella

VII. LACTOBACILLACEÆ

Generic characters mainly those of family..1. Lactobacillus

VIII. BACILLACEÆ

Aerobic, usually saprophytic; cells not greatly enlarged (if at all) at sporulation.1. Bacillus
Anærobic, often saprophytic; cells frequently enlarged at sporulation.........2. Clostridium

Nomenclature

It is most important that each kind of bacterium should have a definite name. The name should be a *binomial* and not a *trinomial.* It is also very desirable that all bacteriologists should adhere to the rules that govern botanists in these matters. Probably the most important points to remember are: To use Latin names for all groups; to recognize only one valid designation for each organism or group and that the oldest (with certain limitations); to designate orders with the ending *ales*, families with the ending *aceae*, sub-families with *oideae*, tribes with *eae*, and sub-tribes with *inae*; to use generic names as substantives and write them with a capital letter; to designate all species by the name of the genus and a specific name or epithet, usually of the nature of an adjective (the two names forming a binomial or binary name).

Relationship of Bacteria*

There has been a great deal of discussion as to whether bacteria are plants or animals. They were first described as animalcula and to the popular mind they are usually animals or "bugs." It is difficult to determine their exact relation philogenetically. These difficulties are so great that some scientists, as Haeckel, would create a new kingdom, call it *Protista*, and put in it some of the lower plants and animals which are difficult to classify, together with the bacteria. The bacteria are undoubtedly more closely related to the blue-green algæ than to any other forms of life. They resemble these organisms in form, method of reproduction, and absence of definite nucleus. It is quite impossible to decide, furthermore, whether some forms, such as *Bact. viride* and *Bact. chlorinum*, are blue-green algæ or bacteria. On the other hand, there are some points of resemblance between the bacteria and the protozoa. Spore formation, similar to that among the bacteria, occurs among some of the protozoa. Another point of resemblance is the possession of flagella. Some of the flagellates quite closely resemble the bacteria in many ways, and the *Spirochætæ*, which are usually believed to be bacteria, have been classed as flagellates by eminent protozoölogists.

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* Prepared by W. D. Frost.

Physiologically the bacteria are quite closely related to the fungi, and are frequently classed with them under the term *Schizomycetes*.

ARTIFICIAL CULTIVATION OF BACTERIA*

The introduction of methods of artificial cultivation marks the beginning of the science of microbiology. These methods were developed by Pasteur and Koch and are depended upon by the microbiologist of to-day as the foundation for most of his work. It has been the aim of investigation to discover a more general culture medium. So far it has been impossible to do this, but beef broth, made after a formula suggested by Loeffler many years ago, forms the basis of nearly all of our culture media. This beef broth, or nutrient bouillon, is made by extracting meat free from fat in water, adding a small per cent of peptone, correcting the chemical reaction, clarifying and sterilizing. To this broth various substances are added for special purposes; gelatin and agar, in order to solidify the media, and various sugars and other chemical substances for the purpose of determining the physiological characteristics of various bacteria. One of the difficulties with the present methods of the artificial cultivation of bacteria is the inconstancy of the composition of the media, due to the fact that the extract of beef, the peptone, and other ingredients, cannot be obtained chemically pure. If it should prove possible to use synthetic substances, such as the polypeptids, it would mark a great step in advance, but it is probably quite impossible to devise a single medium upon which all bacteria will grow. Some bacteria, such as those which produce nitrification, refuse to grow on ordinary media containing organic material. The cultivation of bacteria in pure culture is dependent upon isolation, and the method of isolation suggested by Robert Koch in 1880, and known as the plate culture method, has given eminent satisfaction. This method is dependent upon the use of liquefiable solid media, such as gelatin or agar.

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* Prepared by W. D. Frost.

CHAPTER V

FILTRABLE MICROÖRGANISMS*

The terms "filtrable microörganisms" and "filtrable viruses" are used to designate a group of disease-producing microörganisms that are characterized by their ability to pass through ordinary "bacteria-proof" filters. In the past it has been customary to speak of these filter passers as invisible or ultramicroscopic because of the fact that, besides being filtrable, they were, with the single exception of the virus of bovine pleuro-pneumonia, invisible under the microscope and incapable of multiplying in vitro on any of the usual culture media. Recent discoveries indicate that the terms "invisible" and "ultramicroscopic" are incorrect, at least with respect to some members of the group. So long as clear, filtered fluids that gave no visible evidence of life were capable of setting up infectious disease in men or in animals there was some reason for the use of those terms and even for Beijerinck's fanciful conception of a "living fluid contagion." However, the brilliant researches of Noguchi have offered a technique by means of which some of these hitherto invisible viruses have been cultivated outside of the animal body and made visible under the microscope. While some members of this group may indeed be of ultramicroscopic size, there is reason to believe that many of them will eventually be rendered visible through improvements in bacteriological technique.

The characteristics of the filtrable viruses may be best understood by consideration of a typical example, the virus of foot-and-mouth disease. In this disease vesicles form in the mouths and on the feet of infected cattle. The virus is known to be present in the lymph which forms in these vesicles because this lymph will produce typical attacks of foot-and-mouth disease when inoculated into susceptible animals. If now this infectious lymph be diluted with water and passed through a Berkefeld filter the resulting filtrate will be found to be free from all visible microorganisms and in addition the usual culture tests will give negative results. Notwithstanding this apparent sterility,

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* Prepared by M. Dorset.

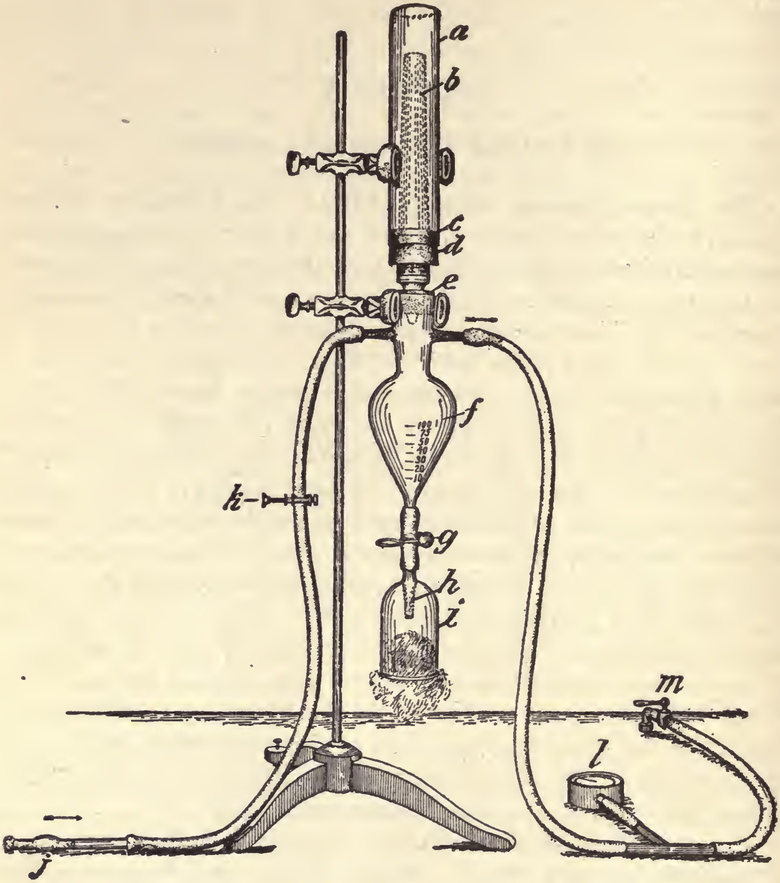

FIG. 90.—Apparatus for fractional filtration, designed for use with Pasteur-Chamberland or Berkefeld filters. *a*, Glass mantle surrounding filter; *b*, Chamberland filter; *c*, paraffin joint; *d* and *e*, rubber stoppers; *f*, double side-arm suction flask; *g*, pinchcock controlling outlet from suction flask; *h*, outlet tube surrounded by glass shield and attached to lower end of suction flask by means of short rubber tubing; *i*, glass shield fused to and surrounding outlet tube as a protection against contamination when the filtrates are drawn off; *j*, glass inlet tube plugged with cotton, for admitting air into suction flask; *k*, pinchcock governing the admission of air into flask; *l*, vacuum gauge; *m*, stopcock connected with vacuum pump. (*U. S. Dept. of Agriculture, Bureau of Animal Industry, Bull.* 113.)

however, the filtrate will produce disease in cattle in the same manner as the unfiltered lymph. It is known that the symptoms produced by the filtrate are caused by a living organism and not by a toxin, because by successive filtrations and inoculations the disease can be transmitted through a long series of animals, thus indicating clearly that there exists in the filtered lymph a living organism which is capable of reproduction. Another proof that the virulence of the filtered lymph is caused by the presence of living corpuscular elements, and that it is not a mere solution of a toxin, is found in the failure of the virus to pass through filters of finer grain than the Berkefeld as, for example, the Kitasato filter. The microörganism of foot-and-mouth disease has not been cultivated nor made visible. Among other diseases produced by filtrable viruses which as yet remain invisible, are hog cholera, rinderpest, swamp fever, fowl plague and South African horse sickness.

The invisibility of this group of microörganisms may depend upon either their minute size or their peculiar structure. The most powerful microscopes will not enable us to discern with distinctness objects which are less than 0.1μ in diameter. We know of bacteria which in size approach this limit quite closely (*M. progrediens*, 0.15μ in diameter) and there is no reason for believing that the size of organisms is limited by our ability to see them. As already stated, invisibility may also result from a peculiarity of structure, such as complete transparency and failure to stain with the reagents ordinarily used for this purpose.

The ability of microörganisms to pass through filters is dependent upon a variety of factors. The size and plasticity of the organism, the fineness of the pores, and the thickness of the walls of the filter as well as the conditions under which the filtration is performed, will all influence the result.

The failure of the filtrable microörganisms to develop under artificial conditions is to be attributed to their strict parasitism and to our inability to imitate exactly in the laboratory the conditions which exist in the animal body. The method of Noguchi, referred to above, and which has done so much to advance our knowledge of the filtrable viruses, was first used to cultivate *Treponema pallidum*. The culture medium is placed in long narrow test tubes and consists of a piece of fresh sterile rabbit's kidney placed in the bottom of the tube over which is poured sterile unheated and unfiltered ascitic fluid or a mixture of ascitic fluid and agar. The surface of this medium is covered with a layer of sterile paraffin oil to exclude oxygen. The material from which cultures are to be made is introduced into the bottom of the tube by means of capillary pipettes.*

While the filtrable microörganisms possess certain qualities in common, in some respects they differ widely from one another. Some will pass only through the coarsest of bacteria-proof filters, while others pass readily through the densest filters, thus indicating wide differences in size or in structure. Some are very susceptible to the action of germicidal agents, whereas others are more resistant than the ordinary bacteria. Some produce disease in only one species of animal, while others show little or no limitation in this respect. The diseases produced by these microörganisms likewise differ markedly, some being comparatively benign and local in character, whereas others appear as the most profound septicæmias. Some are extremely contagious, while others can be transferred from one animal to another only by means of an intermediate host. In fact these invisible microörganisms seem to differ among themselves quite as widely as do those which are visible to us.

The existence of a filtrable microörganism is determined as follows:

The infectious agent must pass through a bacteria-proof filter, which is free from imperfections as shown by tests with visible organisms of small size. Pressure exceeding one atmosphere should not be employed during filtration. The time of filtration should not exceed one hour. The filtrate should remain free from all visible bacteria as shown by microscopic examination and cultural tests. The filtrate should possess the specific disease-producing qualities of the unfiltered material. Animals infected with the filtrate should yield material which, after filtration, will in its turn possess the attributes of the original unfiltered material. Recent suggestive developments have thrown some light on the possible nature of filtrable viruses. The reader is referred to the work of Flexner and Noguchi since 1912, published in the Journal of Experimental Medicine; he is also requested to read the article by Löhnïs and Smith already mentioned on page 99.

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* For details of this method see J. Exp. Med., 1911, et seq.

CHAPTER VI

PROTOZOA*

INTRODUCTION

Many of the diseases which are known to be due to an infecting agent are caused by bacteria; but others are caused by protozoa.

The bacteria belong to the vegetable kingdom. The protozoa are unicellular animals; they are extremely numerous and are very widely distributed in nature. They occur in water, soil and in the bodies of most animals.

From a zoölogical point of view, the protozoa constitute an important sub-kingdom. It is sometimes difficult to say whether a minute organism is a plant or an animal. For this reason, primitive unicellular organisms are sometimes classified by themselves, as *Protista* (pages 11, 117), a kingdom which thus includes not only primitive organisms which have not yet been definitely established in either group but also certain unicellular animals and plants. It appears preferable, however, to determine as far as possible the genetic relationship of various organisms and, by the study of their physiology and modes of development to differentiate between those which are plant-like and those which are animal-like in character. The protozoa are thus included in the animal kingdom and have been defined as "unicellular animals." They are to be distinguished, on the one hand from primitive forms such as bacteria which, lacking differentiation of nucleus and cytoplasm, do not conform to the type of structure of true cells, and on the other hand, from primitive unicellular organisms of plant-like character such as algae and fungi.†

Many protozoa live in fresh water. Others live in the sea; chalk is formed from the skeletons of myriads of protozoa which once lived in the ocean. While a large proportion of the protozoa are free-living, others are parasitic on animals and plants. Some of the parasitic protozoa are practically harmless and do no apparent injury to the hosts which support them; others produce severe diseases. Before mentioning those especially which cause disease (see page 876) it will be well to consider the protozoa as a class and to discuss the characters which all have in common.

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* Prepared by J. L. Todd.

† See page 13.

STRUCTURE OF THE PROTOZOA

Most protozoa are so small as to be visible only by the aid of the microscope but certain species are visible to the naked eye as individuals, or as agglomerated masses of individuals. For example, the *Sarcosporidia*, which occur in the muscles of mice and other animals, can easily be seen without a microscope, and the huge plasmodial masses of *Mycetozoa*, which are sometimes seen on rotting wood or in tan pits, may measure many centimeters in breadth.

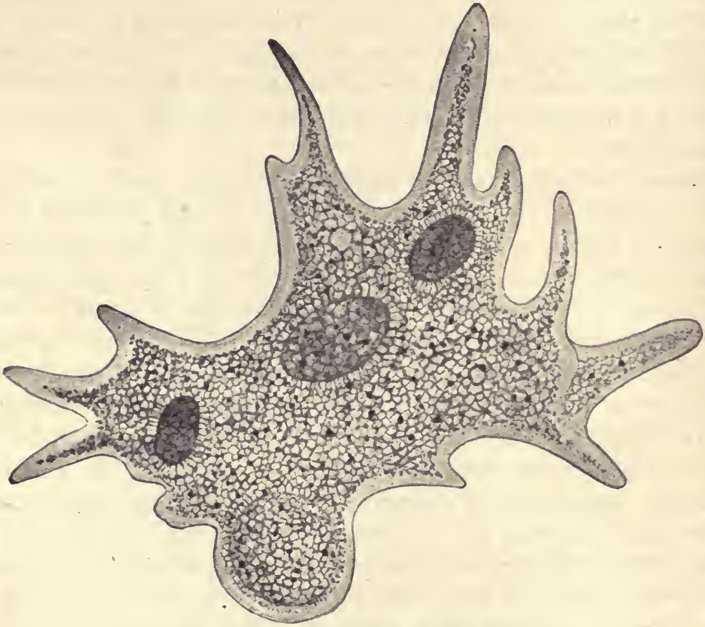

FIG. 91.—*Amœba vespertilio.* (*After Doflein.*)

Like all living things, the protozoa are composed of protoplasm (page 18) and its products. Protoplasm is a complex mixture of various substances in a colloidal condition. When studied by appropriate methods, the protoplasm of a cell appears to be alveolar or foam-like in structure. This is because the protoplasm is emulsoidal in character being composed of a mixture of many more or less non-miscible substances, some of which are fluid in character, others more of the nature of solids. In such a mixture, the more viscid materials form tiny globules, and each of these is surrounded by a layer of softer material (Fig. 91). Consequently, cytoplasm is alveolar in structure; it has an appearance similar to that produced by the myriads of bubbles in a mass of foam. The walls of the outer layer of alveoli, or of alveoli which surround a resistant structure within the cell, are perpendicular to the surface against which they lie but the outline of the alveoli, which are not in contact with a firm structure, is more nearly circular. An exactly similar arrangement of the alveoli may be seen in a mass of soapsuds contained in a bottle; wherever the bubbles touch an unyielding surface, their outline becomes rectangular.

Recent studies in colloidal chemistry and in the microscopic dissection of cells have furnished valuable contributions to the knowledge of the chemical and physical properties of protoplasm. The view has been advanced that protoplasm consists largely of material in a state known in colloidal chemistry as a *gel*, some portions being firm and viscid and others very soft in character. Procedures which convert such material into a *sol* or fluid state are said to cause the protoplasm to quickly disintegrate. Certain portions of the cell such as the limiting membrane, the nuclear membrane and the nucleolus are of firmer consistence than other portions, and some cells contain globules and granules of various types.

The protoplasm of a protozoön may be divided into two main portions: the *cytoplasm* and the *nucleus* (Chapter I). The cytoplasm, as a whole, may be divided, more or less easily, into a clearer, denser, more resistant outer layer—the *ectoplasm;* and a more fluid, granular, internal portion—the *endoplasm*. Denser, more resistant fibers sometimes run through the cytoplasm and, like a skeleton, serve to fix the shape of the organism in which they exist.

The nucleus, in its simplest form, is a structure which is differentiated from the remainder of the cell by being more refractile and by being colored more deeply in specimens which have been stained by dyes. It stains deeply because it contains a substance called *chromatin*. The chromatin usually occurs in granules which may vary considerably in size and which are supported upon a *linin* framework that does not stain by ordinary methods. The interstices of the nucleus are filled with nuclear sap. A limiting nuclear membrane may be present, but it is not an essential part of the nucleus. The nuclear material may be all gathered together in a single mass, or it may be distributed in small granules termed *chromidia* so that, at the first glance, no nucleus seems to be present. Such chromidia may be said to constitute a distributed nucleus, although the term nucleus is usually applied to a well differentiated cell structure.

The nucleus (page 15) is to be regarded as the most important unit in the structure of the cell and is apparently essential for the continued existence of the latter. If cells are divided portions containing no nucleus invariably die while portions containing the nucleus may continue to live and eventually recover from the injury. The rôle of the nucleus is not fully understood but it seems certain that it is a controlling center for the cell's activities. It is concerned in the nutrition of the cell, frequently nuclear structures have to do with the motility of cells and the chromatin serves as a medium for the hereditary transmission of specific characteristics. Its functions, therefore, are at least three-fold since it is active in trophic, kinetic and reproductive capacities. Usually, all these functions are subserved by a single nucleus; sometimes, however, as in the flagellates and many ciliates they are divided between two nuclei (page 18).

Activities of the Protozoa

The higher animals or *Metazoa* are composed of a great number of cells. A protozoön consists of a single cell. In the former the various functions of the body are each carried out by a special type of cell; for example, movement is performed by the muscle cells, digestion is provided for by the cells of the alimentary tract, and urine is excreted by the kidney cells. A protozoön being a unicellular animal, these various functions must be performed within the single cell of which it consists. Consequently certain parts of its protoplasm are especially differentiated and function in a manner similar to the organs of multicellular animals. Such differentiated parts are termed *organellæ* and by means of these the protozoa move about, feed, and excrete waste products in many respects like the higher animals.

The activities of a protozoön may be considered under LOCOMOTION, METABOLISM* and REPRODUCTION.

LOCOMOTION.—The protozoa have several different modes of moving themselves about. Some of them move by the formation of temporary processes or *pseudopodia;* in this method of progression, the protoplasm flows out, in finger-like processes, from the body of the organism and, as the protoplasm flows into these processes, the whole organism progresses, literally, by flowing along. Some of the gregarines move about by means of a flowing of the protoplasm which always takes place in one direction; it is probable that the control of the direction of the flow in these parasites is effected by the contraction of *myonemes.* These are contractile fibers, which usually lie near the surface of the organism possessing them. Through their contraction, the form of the body of the parasite may be altered and, in this way, motion may be produced. *Cilia* are small hair-like processes, which may occur either in definite areas or in large numbers over the whole surface of a protozoön. They produce motion by waving and, acting together, make a strong simultaneous stroke in one common direction. The movement of all the cilia of an organism is, however, usually not synchronous but proceeds in waves across the surface of its body so that the appearance is similar to that produced when a breeze passes across a field of grain. *Flagella* are larger than cilia; they are whip-like processes which have a lashing movement. They are usually few in number and are often placed at the ends of the organism *Undulating membranes* consist either of a thin fold of the surface layer or of rows of fused cilia and form either fin-like organs ex-

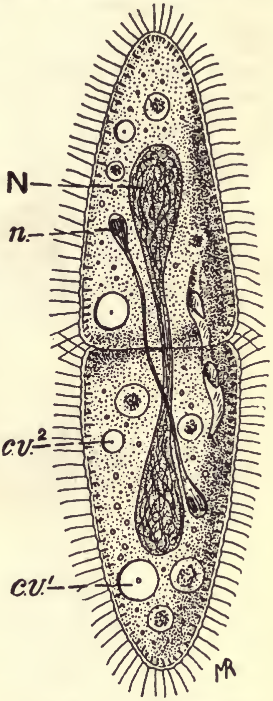

FIG. 92.—*Paramecium caudatum:* division showing the macronucleus (N) dividing without mitosis, the micronucleus (*n*) dividing mitotically. $c.v^1$., Old, and $c.v^2$., new, contractile vacuoles. (*Minchin, after Bütschli and Schewiakoff, in Leuchart and Nitsche's Zoologische Wandtaflen, No.* LXV.)

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* (See p 195.)

tending along the surface of the organisms or special organs for the intake of food.

REPRODUCTION

The protozoa reproduce in many different ways and several of these ways may occur in a single organism. For this reason, their reproductive power is very great; in power of repeating their like, they fall just short of the bacteria. The union of a male and a female form does not always precede multiplication; sexual union and reproduction, though now combined in many animals, may have been originally two entirely distinct phenomena and, in the protozoa, though sexual union may be concerned with the production of new individuals, it is often especially associated with the regeneration of the protoplasm of the parasites taking part in it.

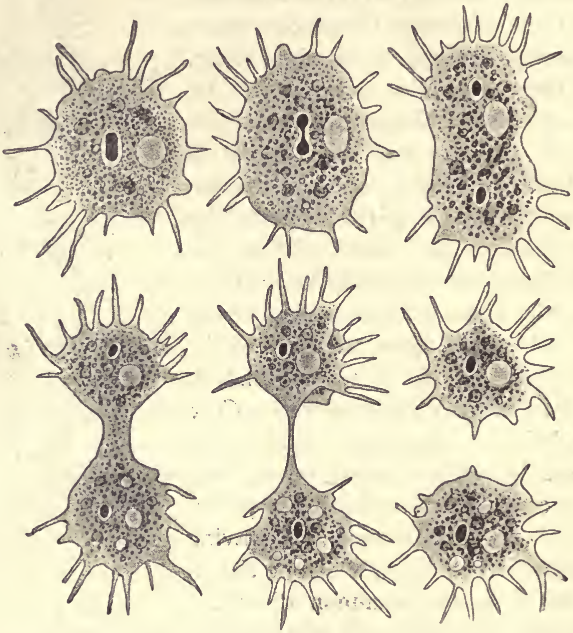

FIG. 93.—Stages in the division of *Amœba polypodia.* (*After F. E. Schulze and Lange from Doflein.*)

The simplest of the methods of reproduction is simple *binary division,* in which the organism divides into two equal parts. A modification of this process is *gemmulation,* in which a small protozoön buds off from a larger parent; sometimes many buds are formed rapidly, one after the other, until the parent protozoön disappears in a swarm of daughter cells. When a protozoön divides at a single division to produce a large number of daughter cells simultaneously, the process is called *schizogony* and the young parasites are called *merozoites*, if a sexual fertilization has not immediately preceded the act of division; if such a division, in which the parent organism disappears, takes place after a fertilizing act, the process is called *sporogony* and the young parasites are *sporozoites*.

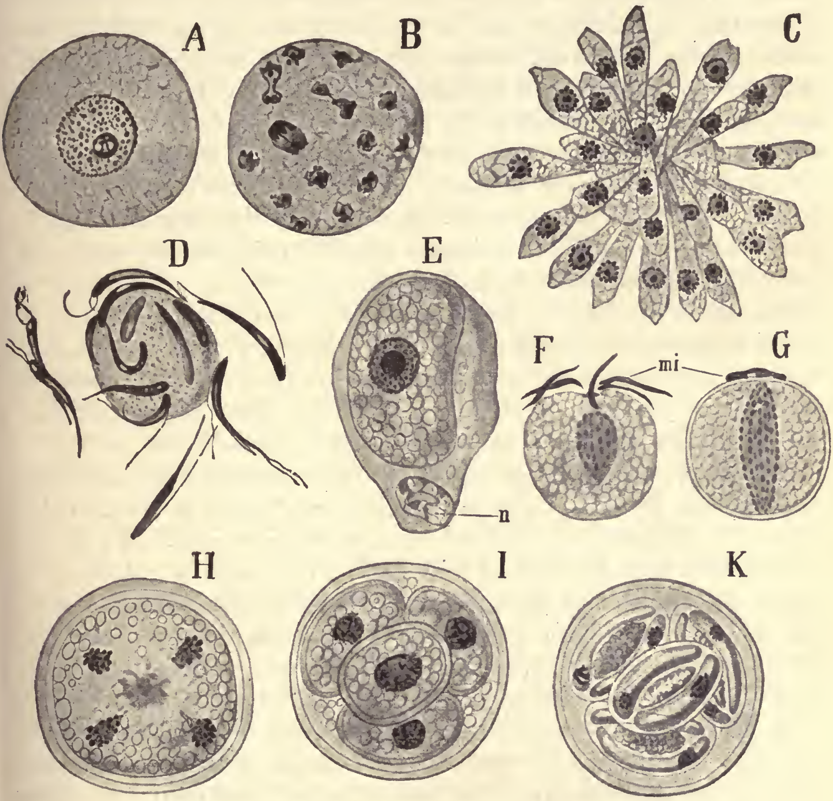

FIG. 94.—*Coccidium schubergi.* *A–C*, asexual multiplication; *D–K*, sexual multiplication; *D*, microgametes; *E*, macrogamete; *F*, *G*, fertilization; *H*, *I*, *K*, division and spore production. (*After Schaudinn, from Doflein.*)

In protozoa, as in metazoa, the essential process in fertilization is the union of two nuclei of opposite sex. In dividing, cells may go through a process called *mitosis* during which the chromatin of the nucleus is grouped into more or less rod-shaped masses which are called *chromosomes.* The number of chromosomes which are formed during mitosis is constant and characteristic for each species. In the reproductive areas, during the two divisions just preceding the maturity of cells which are to become ova or spermatozoa, the number of chromosomes is reduced to exactly one-half of the number which are formed during the division of cells outside of the reproductive areas of the same animals. The process by which the number of chromosomes is reduced to one-half is termed *chromatic reduction,* and the fragments of chromatin which in the female are unused and which are extruded from the cell during the process are called *polar bodies.* While reduction in the number of chromosomes has been shown to occur prior to fertilization in a number of the protozoa, in many species a more primitive process consisting of the mere extrusion of masses of chromatin irrespective of the number of chromosomes is found to occur. It is evident that the chromatin is, at least usually, reduced in amount preparatory to the sexual process.

Although in certain of the protozoa nuclear division is accomplished by a process of mitosis similar to that which occurs in multicellular animals, in many it is affected by a much more primitive process. The nucleus may be resolved into scattered granules of chromatin—chromidia—which may subsequently become reconstructed into a number of nuclei. The nucleus may divide by direct division, that is, by simple constriction into two approximately equal parts. Between this form of division and the classical mitosis there is every possible transition. The centrioles or centrosomes are frequently intranuclear in the protozoa. In the case of primitive nuclei without definite nuclear membrane a division simulating mitosis is termed *promitosis.* In other forms in which there is a nuclear membrane but in which the centrioles remain intranuclear throughout division, the process is called *mesomitosis.* The nuclear membrane often persists throughout division and the chromosomes are in many forms very minute or are not definitely formed.

The fertilizing processes which occur in the protozoa may be grouped under three heads: *Copulation, Conjugation* and *Self-fertilization.* In *copulation* two whole cells unite. The cells taking part in this union are called gametes and there are the male or *microgametes*, and the female or *macrogametes*. The cells which produce the gametes are called *gametocytes*. The product of the union is called a *copula* or *zygote*. If the uniting cells be equal in size the copulation is *isogamous;* if they be unequal, the copulation is said to be *anisogamous*. Anisogamous copulation, the union of two unequal cells, is most typically seen in the fertilization of a large macrogamete by a small microgamete. *Copulation* is the most common fertilizing process among the pathogenic protozoa. *Conjugation*, the second method of fertilization, only occurs among the ciliata. In it, two adult individuals place themselves in apposition. The nucleus of each cell first reduces and then divides into two halves, one male, the other female. Each organism retains its female half nucleus, while an exchange of the male half nuclei is effected. Processes of *self-fertilization*, such as *autogamy* and *parthenogenesis*, are included under the third heading. In *autogamy* the nucleus of a single cell divides into two parts. Each of these may undergo further division, during which the chromosomes are reduced or there may be a simple extrusion of a portion of the chromatin. The two resulting, reduced nuclei then unite, in the same cell, to form a new nucleus. *Parthenogenesis* is the development of new individuals from a female cell without a preceding fertilization; this process possibly occurs in many protozoa, and through it perhaps may be explained the reappearance of malaria in patients who once suffered from that disease and were thought to have recovered.

The LIFE CYCLE of a protozoön consists of the changes through which it passes in the period intervening between each fertilizing act. In many of the pathogenic protozoa, an alternation of generations occurs; that is, cycles of development in which an asexual method of reproduction occurs, alternate with cycles of development in which reproduction is effected by sexual methods. The developmental cycles are commonly punctuated by binary or multiple division, by encystment, and by transference to a second host as a necessary factor for the completion of the life cycle. An alternation of generations occurs in the life cycle of one of the most important of the pathogenic protozoa, the parasite which produces malaria (Fig. 189). While it is in the body of its mammalian host, man, it multiplies through multiple fission or schizogony; the sexual, or propagative phase of its development occurs within the body of its invertebrate host, a mosquito. The host in which the adult, sexual stages of the parasite occur, in this instance the mosquito, is said to be the *definitive host;* hosts harboring the parasite while it is in other stages are called *intermediate hosts.*

ENCYSTMENT.—Under unfavorable conditions, such as dry surroundings, many protozoa are able to surround themselves by a resistant cyst and to enter upon a resting stage of indefinite length. The cyst protects them from harmful influences and, surrounded by it, they remain in a resting state until favorable circumstances come about once more. The power of forming resistant cysts plays an important part in the life history of many parasitic protozoa; it is especially so with those protozoa which have become so specialized that multiplication or continuous existence independent of their appropriate host has become impossible for them. It is often through the formation of cysts that an infection by a protozoön is spread, and, as in the coccidia (page 889), the presence of such a stage is often absolutely essential in the life history of a parasite.

PARASITISM

A parasite is an organism which is, at some time, directly dependent upon another, usually, a larger organism.

Although the word parasite is often used as though it referred only to organisms belonging to the animal kingdom, parasites may be either animal or vegetable; bacteria and fungi, which live at the expense of other living beings, are parasites just as the disease-producing protozoa and the biting insects which transmit them are parasites.

Most parasites are simple organisms, low in the scale of life. They nourish themselves without exertion, at the expense of their hosts, and as might be expected, their unemployed organs, such as the sensory locomotory and seizing appendages, by means of which food is usually obtained, gradually disappear; degeneration always occurs in an organism which assumes a parasitic mode of life.

Organisms, such as the malarial parasite, which are wholly dependent for existence upon their hosts, are called *obligatory* parasites; those which are not, such as the infusoria usually found in the stomach of herbivorous animals, are *facultative* parasites. Facultative parasites often feed upon organic material provided by the host, and not upon the host itself; but they are capable of living indefinitely apart from the host.

If an organism is attached to a host, and neither harms nor benefits it, such an organism and its host are said to be *commensals*. For example, the spirochætes found about the teeth of many persons are usually harmless; they are commensals of their host. When the host of an obligatory parasite dies, the parasite often perishes also. Consequently, it is contrary to the interest of such a parasite to destroy its host; yet parasites often do harm their hosts. The harm done by a parasite to its host is the disease which that parasite causes. Disease is recognized by symptoms. The nature of the symptoms depends directly upon the nature of the harm done by the parasite. The symptoms are the result of interference by the parasite with tissues, or the functions of tissues, in the host. The pathogenic protozoa may injure their hosts in at least three ways: They may feed upon, and destroy cells; they may produce poisonous toxins; and their presence may do damage by mechanically obstructing some of the functions of its host. All three of these ways are well exemplified by the action of the malarial parasite in man (page 892).

DISCUSSION OF THE CLASSIFICATION*

The following grouping of the *Protozoa* gives a general idea of the position, in zoölogical sequence, of the individual parasites which are spoken of in the subsequent pages. The *Protozoa* are here grouped in four classes: the RHIZOPODA, the FLAGELLATA, the SPOROZOA, and the INFUSORIA; and these classes are divided directly into genera. This is by no means a complete classification of the protozoan families. Many orders, families and genera are unmentioned because they are parasitic neither in man nor in animals; and of the organisms mentioned, only those which are constantly causes of disease are described.

The form of a protozoön may vary greatly at different stages of its development; for example, the adult herpetomonas is an active organism moving by means of a flagellum, quite unlike its spherical form which is without a flagellum. Consequently, the whole life history of a protozoön must be known before it can be classified with absolute certainty. The whole of the life history is known for only a few protozoa; and, though the organisms mentioned in this classification are placed in the position usually given to them, it must be understood that this classification is not final, and that the discovery of new stages in the life history of some of these protozoa may make it necessary to remove them from the classes in which they have been placed. For example, before its flagellate stage was known, *Leishmania donovani* was classified with the sporozoa; now it is grouped with the herpetomonads.

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*(See p. 13.)

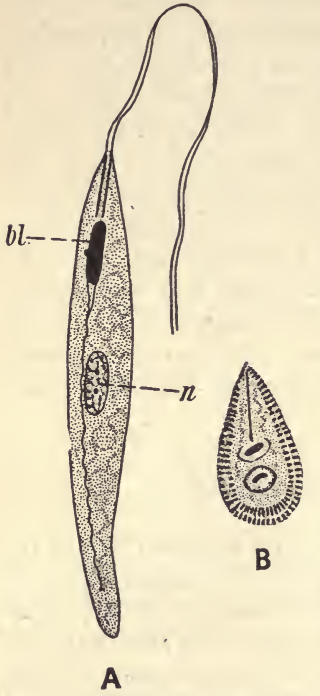

Fig. 95.—*Herpetomonas muscæ-domesticæ* (Burnett). *A*, Motile individual with two flagella; *B*, cyst; *n*, nucleus; *bl*, kinetonucleus. (*After Prowazek from Minchin.*)

The characteristics of the different genera and of the unimportant parasites are very briefly mentioned in the following paragraphs; the important parasites are treated more fully in the pages indicated by the references given, in brackets.

The RHIZOPODA include the simplest forms of animal life. A rhizopod, such as an amœba, consists of a single cell, without a protective covering, and without permanent organs of locomotion; it moves about and captures its food through the agency of its pseudopodia. Very few of the rhizopods are parasitic; most of those which are parasitic, belong to the genus *Entamœba*. Different species of parasitic amœbæ may occur in the alimentary canals of various animals. Certain of these produce serious diseases (page 876).

The FLAGELLATA are distinguished by possessing one or more flagella; they often have, also, a fin-like, undulating membrane extending along the surface of their body. Many possess two nuclei, a larger trophonucleus which has to do with nutrition and a smaller kinetonucleus which is intimately connected with the organs of locomotion. This group has been termed the *Binucleata* by certain systematists. Most flagellates are free-living. Comparatively few species are parasitic, but some of these cause very serious diseases (page 879).

A *Herpetomonas* is an elongated organism which possesses trophonucleus and kinetonucleus. The latter is situated near the flagellar or anterior end of the parasite, and from it arises a terminal flagellum. A *Herpetomonas* has no undulating membrane. A *Crithidia* is an organism like a *Herpetomonas*, but possessing an undulating membrane. A *Trypanosoma* is an elongated parasite which has a trophonucleus, a kinetonucleus usually situated near its aflagellar extremity and an undulating membrane along the border of which the flagellum extends to terminate in a whip-like appendage. Species of *Herpetomonas*, *Crithidia* and *Trypanosoma* are frequently found in the intestines of insects. One species of *Herpetomonas* is a frequent and harmless parasite in the intestine of the house fly. Many serious diseases are caused by trypanosomes. The genus *Trypanoplasma* includes organisms which have a flagellum at either end, as well as an undulating membrane. They are parasitic in the blood of fishes. The genera *Cercomonas*, *Monas*, and *Plagiomonas* include small, unimportant flagellate

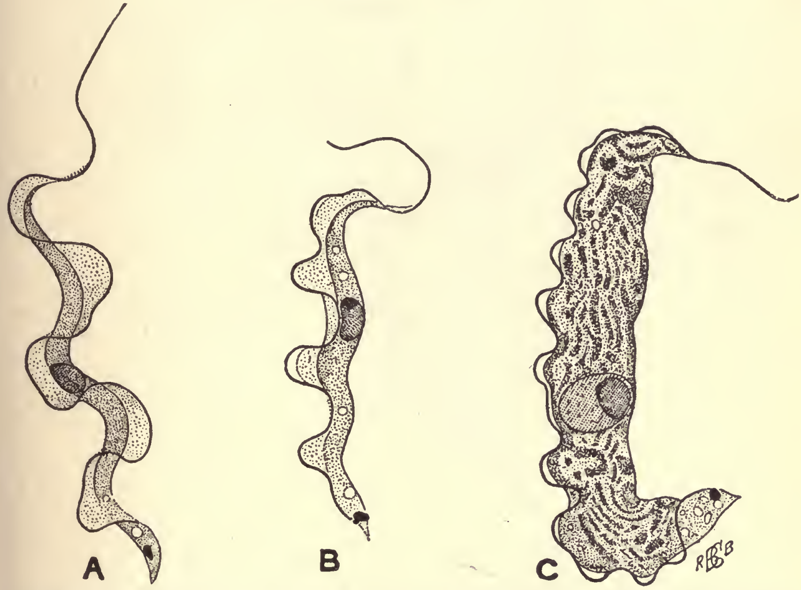

FIG. 96.—*A*, *Trypanosoma tincæ* of the tench; note the very broad and undulating membrane in this species; *B*., *C*., *T. percæ* of the perch, slender and stout forms. (*After Minchin*, × 2000.)

organisms which have been found, occasionally in the human intestine and vagina, and in necrotic material from the lungs. *Trichomonas* is a pear-shaped organism which has four flagella attached to its blunt end, and an undulating membrane extending from the origin of the flagella at the anterior end posteriorly over the surface of its body.

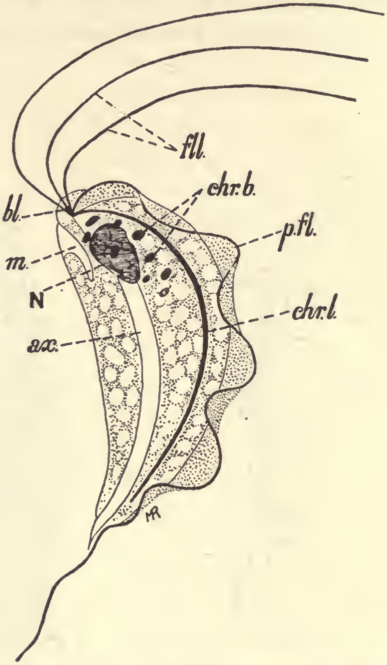

FIG. 97.—*Trichomonas eberthi*, from the intestine of the common fowl; *fll.*, anterior flagella, three in number; *P.fl.*, posterior flagellum, forming the edge of the undulating membrane; *chr. l.*, "chromatinic line," forming the base of the undulating membrane; *chr.b.*, "chromatinic blocks;" *bl.*, blepharoplast from which all four flagella arise; *m.*, mouth opening; *N.*, nucleus; *ax.*, axostyle. (*From Minchin, after Martin and Robertson.*)

One of the four flagella is usually directed backwards and extends along the border of the undulating membrane. One species is sometimes found in the human bladder. Other species are common, usually harmless, parasites in the intestines of pigs, frogs and other animals. The most important species of the genus *Lamblia* is *Lamblia intestinalis.* It also is a pear-shaped organism. It has several flagella and is distinguished by possessing a depressed sucker, by which it attaches itself to the intestinal epithelium of the animal in which it lives. It is a cause of diarrhoea in man, and also of a fatal disease of the intestines in rabbits; but it is almost invariably found in the duodenum and first portion of the small intestine of normal laboratory animals such as mice, rats, and rabbits.

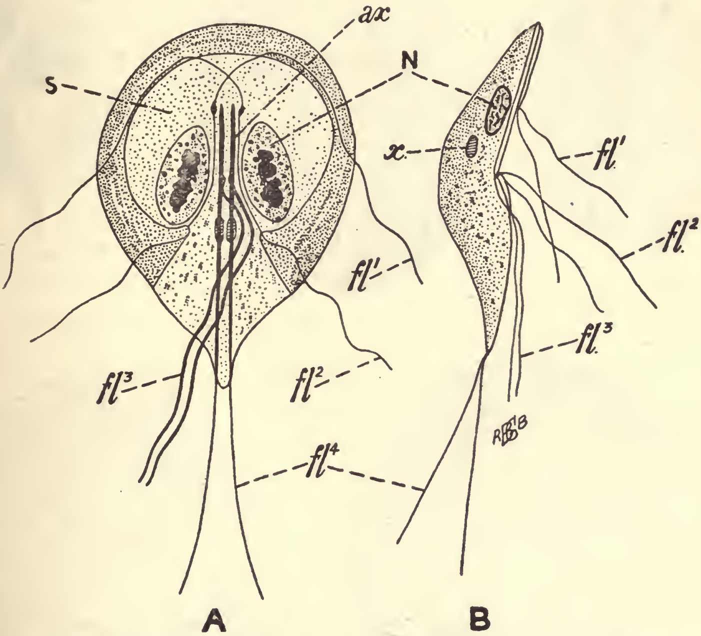

FIG. 98.—*Lamblia intestinalis.* *A*, Ventral view; *N.*, one of the two nuclei; *ax.* axostyles; *fl.*[1], *fl.*[2], *fl.*[3], *fl.*[4], the four pairs of flagella; *s.*, sucker-like depressed area on the ventral surface; *x.*, bodies of unknown function. (*After Wenyon* (277) *from Minchin.*)

The SPOROZOA are parasitic protozoa which multiply by the production of spores at some stage of their life cycle. There are very many sporozoa and so, for convenience of classification, they are subdivided into seven orders. The *Gregarinæ* have a very distinctive shape; the single cell, of which they are composed, is divided into two or more divisions. The first of these divisions is furnished with hooks or other structures through which the parasite attaches itself to its host. None of the gregarines are parasitic on mammals; worms are the hosts for some of them. The *Coccidia* are usually parasitic within certain cells of their host, for example, *Coccidium stiedæ* (*Eimeria cuniculi*) (page 889) enters the epithelium of the small intestine and of the bile ducts of the rabbit, while *Eimeria avium* enters and destroys the cells lining the intestines of the birds which it infects (page 889). The *Hæmosporidia* live, for a part of their life cycle, within the red cells of the blood of

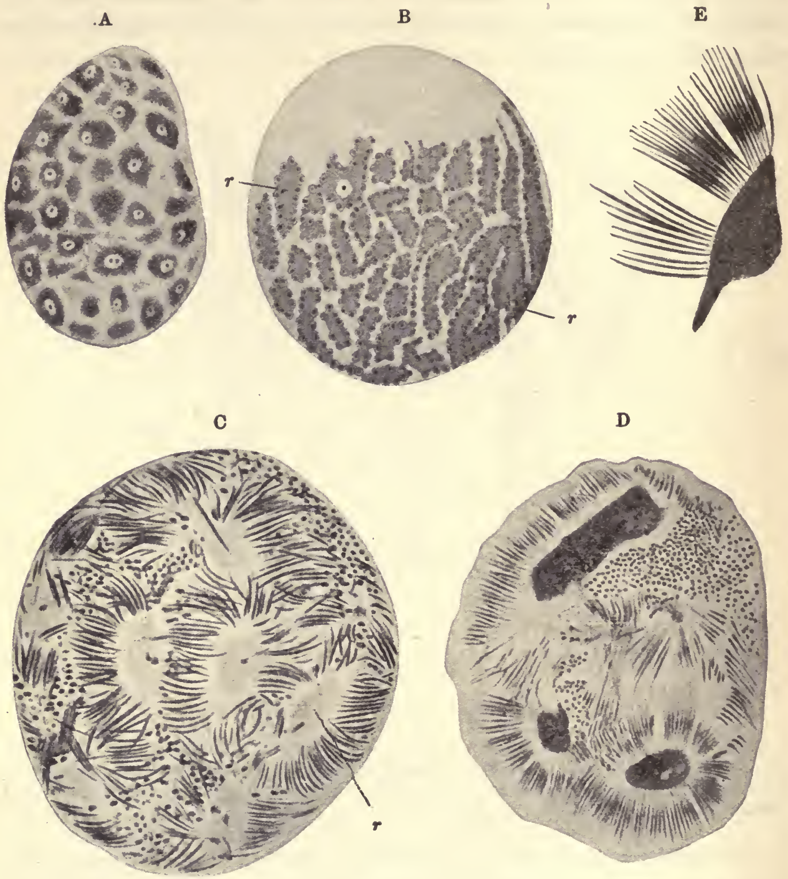

FIG. 99.—Sporozoits in the oocyst of *Laverania malariæ*. *A*, Formation of nuclear points which serve as the foci from which the sporozoits develop; *B*, a more definite shaping of protoplasm and nuclei; *C*, *D*, mature sporozoits in the oocyst arranged about centers from which they radiate; *E*, a portion of one enlarged. (*After Grassi, from Doflein.*)

vertebrate animals. They are a very important order. The genus *Plasmodium* causes *malaria in man* (page 890); while *Proteosoma* and *Hæmoproteus* are malarial parasites of birds (page 890). The *Hæmogregarinæ* are usually harmless parasites of reptiles and batrachians (frogs); a part of their life is passed within the red cells of their host, but they have a slowly moving stage, somewhat resembling a gregarine, which occurs free in the blood. *Hepatozoön perniciosum* is the best known of a group of hæmogregarine-like parasites which are parasitic, often within the white cells of the blood, in dogs, in rats, and in other rodents; so far as is known, they do not cause disease. The genus *Babesia* (page 894) includes parasites which cause important diseases in cattle, sheep, horses and dogs. Similar parasites have been found in the blood of monkeys, of dogs, of rats and other rodents. The *Sarcosporidia* are tube-like in shape and filled with spores. They are found within the cells of the voluntary muscles. The *Haplosporidia* are a group of very small sporozoa of which little is known. Some of them are parasitic in fish; one of them, *Rhinosporidium kinealyi*, has been found in a tumor of the nose of a native of India. The *Myxosporidia* (page 899) are recognized by the peculiar form of their spores; each spore has one or more capsules each furnished with a coiled filament or thread which is extruded under certain conditions and probably serves to anchor the spore to a surface upon which further development may occur. Members of this order are parasitic in various tissues of fishes and they often produce disease in their hosts. The spores of the *Microsporidia* (page 899) are exceedingly small; a member of this order is the cause of *pébrine* in silk-worms (page 937).

The INFUSORIA (page 899) are a large class. Most of them are not parasitic. They are the most highly developed of the protozoa and their bodies are more or less covered with cilia, by which they move themselves through the liquids in which they live.

Lastly, under the heading *Parasites of Uncertain Position*, are grouped a number of organisms which cannot be classified because so little is known of them at present. The spirochætiform organisms, *Histoplasma capsulatum* (page 900), the *Chlamydozoa* (page 900), the *Rickettsias*, and the *Ultramicroscopic viruses* (page 119) are all associated with important diseases in men and in animals.

The SPIROCHÆTÆ (page 900), as their name signifies, are thread-like organisms, which seem to be coiled in a spiral. It is probable that the curves of certain spirochætes lie in one plane and, consequently, that their bodies are really waved and not spiral. These organisms have no organized nucleus. The chromatin is distributed throughout their bodies.

Those parasites which are important enough to require special consideration are described (page 876) in the order in which they are mentioned in the classification (page 13). Whenever it is possible to do so, a single species is taken as the type of each genus and that species, with the disease it produces, is described; if the remaining species of the genus are mentioned, they are spoken of only to indicate how they differ from the description of the type.

TECHNIC*

The methods employed in studying the pathogenic protozoa are very similar to those used in bacteriology. Microscopes, with the highest magnifications, are essential for successful work.

It is of great importance in the study of protozoa to examine them in the living condition. In no other way can their mode of locomotion be determined and frequently their contour is quite different in living and in fixed preparations. A small amount of the material in which they occur may be placed beneath a cover-glass on a clean slide and examined immediately with the microscope by ordinary daylight. In case large organisms are examined in rather thin fluid it is well to prevent their being crushed by interposing several minute globules of paraffin between slide and cover-glass. This is readily accomplished by touching paraffin with a hot needle and transferring it thus melted to several points on the slide before the preparation is made. When very minute forms are to be studied it is necessary to utilize what is known as the dark field illumination. This brings out very minute organisms and particles which, being transparent, are invisible to ordinary transmitted light. The dark field apparatus consists of a strong source of light such as a small arc lamp, a special condenser which deflects the light so that objects in the microscopic field are illuminated by light directed from the sides, causing them to appear bright on a dark background. Another method of obtaining a dark field is to mix on a slide a small drop of the material to be examined with an equal-sized drop of India ink, or better of saturated aqueous solution of nigrosin, and then to smear this mixture across the surface of the slide. It is then dried and examined at once by the oil immersion lens. Only ordinary daylight is required for this method but it does not serve in the study of the motility of organisms.

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*For more detailed instructions for the study of protozoa see Fantham, Stephens and Theobald, The Animal Parasites of Man, William Wood & Company, New York; Castellani and Chalmers, Manual of Tropical Medicine, Bailliere, Tindall & Cox, London; Stitt, Practical Bacteriology, Blood Work, Parasitology, Blakiston, Philadelphia; Brumpt, Precis de Parasitologie, Masson, Paris; Langeron, Precis de Microscopie, Masson, Paris; Doflein, Lehrbuch der Protozoenkunde, Gustav Fischer, Jena; and Prowazek, Der mikroskopischen Technik der Protistenuntersuchung, Leipzig.

By special apparatus it is possible after obtaining a certain amount of skill to dissect many forms of protozoa. In this way knowledge is obtained of the physical properties of various portions of their bodies and it is also possible to inject various chemicals into their substance. This method of study is made possible by the mechanical devices utilized by Barber to whose work the reader is referred.*

In order to make stained preparations the material may be either smeared in a thin film upon clean slides or sectioned after appropriate treatment. In each case the material requires fixation. For the preparation of stained smears the Giemsa method is widely used. This is briefly as follows:

1. Make thin smears of material on a clean and dry slide.
2. Fix immediately by covering the smear with pure methyl alcohol which should be allowed to act for ten to twenty minutes.
3. Dry by waving slide to and fro.
4. Stain for four to twenty-four hours, according to the depth of stain desired, in a solution made by an addition of one drop of Giemsa stain to 1 c.c. of distilled water.
5. Rinse with distilled water.
6. Dry and mount in immersion oil or any acid-free balsam.

It is frequently desirable to keep stained smears unmounted as they apparently retain their color for a longer period of time. They may be studied with the oil immersion lens but the oil should at once be rinsed off with xylol, for if left upon the preparation an insoluble substance is formed which produces a clouded appearance. All stained preparations should be stored away from the light when not in use. For the above method it is important to have all glassware perfectly clean and without trace of acid. The stain must be used immediately after preparation. Certain materials may be smeared very readily with the platinum loop ordinarily used in bacteriology. A very practical method for making blood smears is to gather a minute drop of freshly drawn blood from a small needle-prick of the skin on one edge of the end of a slide. The latter is placed in contact with the surface of another slide and being held at an angle of 45 degrees is pushed steadily lengthwise across its surface. By increasing or decreasing this angle a thicker or thinner film may be made. Certain investigators prefer to use what is termed the wet method for the fixation of smears. In this case the smear is dropped face down immediately and before drying into a fixative composed of two parts of a solution of saturated $HgCl_2$ in distilled water and one part of absolute alcohol. The technic employed in the staining of sections is then followed and the smear is not allowed to dry at any step in the procedure.

The preparation of stained sections requires a considerable amount of technical skill. Tissue is first fixed to render its structure permanent. It is then dehydrated in alcohol of increasing strengths, next placed in chloroform or some other clearing reagent and it is then imbedded in paraffin, after which it may be sectioned. For the details of sectioning and the staining of sections the reader is referred to Mallory and Wright's Pathological Technic, W. B. Saunders and Co., and to Lee's Vade mecum.

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* Barber: University of Kansas, Science Bulletin 1907-4-3; also Journal of Infectious Diseases, 1911, 8, 248, and 1911, 9, 117.

The cultivation of free-living protozoa is usually accomplished by keeping a supply of the medium in which they live on hand. Hay infusion prepared by boiling a quantity of chopped hay in water is an easy and valuable method of preparing culture media. For the cultivation of amœbæ, the following media is widely employed. It should be noted, however, that the amœbæ which have been cultivated are regarded as free-living forms and the attempts to cultivate parasitic amœbæ have thus far been unsuccessful.

Medium of Musgrave and Clegg

Agar	20 to 30 g.
Liebig's extract of beef	.3 to .5 g.
Common salt	.3 to .5 g.
Water	1,000 c.c.

This medium is designed to provide for slow bacterial growth in order to provide food for amœbæ. On a richer medium the latter are overwhelmed by the rapid growth of bacteria.

For the cultivation of trypanosomes, leishmania and other flagellates the so-called triple N media is employed. This is prepared as follows:

Nicolle, Novy, MacNeal Medium

Water	900 c.c.
Salt	6 g.
Agar	16 g.

Dissolve, distribute in tubes, sterilize and add to the medium in each tube after liquefying and cooling to 40°–50°C. one-third its volume of rabbit blood obtained by cardiac puncture. Slope the tubes for twelve hours, incubate at 37° for five days to prove the sterility of the medium and then keep them at the ordinary temperature of the laboratory for a few days before sowing them. (The tubes should be sealed to prevent evaporation.)

The malaria organisms have been made to continue development outside the body by the following method devised by Bass.

Bass's Method.—The blood in 10- to 20-c.c. quantities is taken from the patient's vein and received in a centrifuge tube which contains $\frac{1}{10}$ c.c. of 50 per cent. glucose solution. A glass rod, or piece of tubing, extending to the bottom of the centrifuge tube is used to defibrinate the blood. After centrifugalizing there should be at least 1 inch of serum above the cell sediment. The parasites develop in the upper cell layer about $\frac{1}{50}$ to $\frac{1}{20}$ inch from the top. All of the parasites contained in deeper lying red cells die. To observe the development, red cells from this upper $\frac{1}{20}$-inch portion are drawn up with a capillary bulb pipette.

Should the cultivation of more than one generation be desired, the leucocyte upper layer must be carefully pipetted off, as the leucocytes immediately destroy the merozoites. Only the parasites within red cells escape phagocytosis. Sexual parasites are much more resistant, and the authors think they observed parthenogenesis. The temperature should be from 40° to 41° and strict anaerobic conditions observed. Æstivo-autumnal organisms are more resistant than benign tertian ones. Dextrose seems to be an essential for the development of the parasites.

Noguchi first cultivated spirochætes; others have extended and modified his work. If a few drops of blood containing spirochætes is dropped into sterile ascitic fluid, hydrocele fluid, or blood plasma, to which a piece of sterile tissue, such as rabbit's kidney, is added, the spirochætes multiply. The test tube should contain about 15 ccm. of culture fluid. It is advantageous to cover the fluid with sterile paraffin oil.

PART II

PHYSIOLOGY OF MICROORGANISMS

DIVISION I

INTRODUCTION*

Microbial physiology seeks to understand the material or concrete processes and functions of protoplasmic activity which integrate in the phenomenon of life. They are embodied in some form of an organism. The many assembled and harmonious forces involved in a unit of life, while they may be resolved in a degree elementally, are dependent upon the structure, composition, and energy values of the life-form, and also upon its environment; likewise the reverse is true. The concomitant relations of forces to the life-form and life-form to the forces are more or less hidden at present, yet they are slowly becoming apparent.

Owing to the multiplicity and variety of forces operating in physiological functioning, it is patent that physiology is complexly composite in nature and must resort to the elemental branches of science as physics, chemistry and morphology for its understanding and exposition. Shrouded along with demonstrated knowledge is the mysterious veil of life which makes of it a reality, subject to the ready onslaught of scientific attack and to a spirit which halts approach.

Besides the basis of facts found in physics, chemistry and morphology which contribute freely to the structure, there are the immediate matters of cytology, anatomy both gross and histological, and environmental conditions which lead into fields of essential technic, before physiology can be truly grasped or successfully studied. When the study is attempted in an elementary manner it consists in recognizing observational functions without attempting to explain or understand the mechanism behind. Such attempts belong to the first steps in primary and secondary education. The limitations or boundaries of physiological knowledge are those established by the human mind in laying hold of and utilizing the nature and the facts of the elemental studies upon which physiology rests and its ability to translate them in the life-mechanism at work.

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* Prepared by Charles E. Marshall and Arao Itano.

CHAPTER I

THE UNIT OF BIOLOGICAL ACTIVITY*

Whether a cell acts in the capacity of an individual entity or alone, as in the case of *S. cerevisiæ,* or in its intimate association with other cells possessing an individual entity and having an intercellular relationship as yeasts and acetic bacteria, or even in close dependent relationship with other cells in forming a multicellular entity as in the case of metazoa and metaphytes, its self-functioning processes or its performances are confined within the limits of the cell, are the operating mechanism of the cell and are independent of other cells, notwithstanding the controlling influences of environmental conditions upon its activities. The self-contained cell, which is a cellular entity, may not be subject as a rule to the more immediate influence of other cells, yet the associative influence exists in most cases whether near or remote and is more or less essential to the life of the cell. There is, in other words, a biological interdependence in most living forms. It follows that the cell has therefore a distinct life of its own within its sphere of activity and in addition an equally important office to perform in its association with other cells; in both cases, it functions only together with its environment.

The cell is at the mercy of environmental conditions. *S. cerevisiæ* is master of itself until the factors of food, moisture, respiration, temperature and reaction are demanded for the sustenance of life; it then becomes the menial servant of each, for each and every factor is essential to its existence. In turn, food, required gases, temperature, and other environmental factors, such as may be needed for cell life, may have their source in the activities of other cells. Yeast cells produce alcohol for the acetic bacteria as certain organs of the body produce *hormones* for the activity of other organs. One cell, therefore, through external factors may become dependent upon another and probably is in the case of most cells.

Unless the microörganisms which seemingly live directly upon the very simple elements of nature are excluded, cellular life is so intricately bound up with other cellular life that it ceases without such association, whether it is regarded from the standpoint of individual entities, the protophytes and the protozoa, or the standpoint of the complex entities, the metaphytes and metazoa. Virchow made the cell the working unit system of life, but it was done in the sense that the "House of Rothschild" has become a unit in the financial world. Pflüger, Verworn, Ehrlich and Vaughan, however, resolve the cell or the "House" into the ultimate coördinated agencies within, molecular complexes, which are responsible for the inception and continuing of all the activities. There are cells, it is true, of many kinds and different degrees of structure and organization, accordingly a more elemental unit must be sought which is essential to establish harmony or unity in life's ultimate phenomena or reactions. Vaughan, who is the last of the above to write from his own investigations, says: "The cell is not the unit of life; life is molecular. Life is function, not form." Again he says: "Cells consist of a chemical unity made of giant molecules." Moore states that "the unit of the biologists is the living cell," but he himself approaches it from the standpoint of molecular structure. He would impugn the attitude and circumscribe the field of the biologist by the limits of morphology whereas, in fact, the biologist interprets organic life by means of the various ultimate elements included so far as this is possible and endeavors to unify all forces and structures in an intimate unity.

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* Prepared by Charles E Marshall and Arao Itano.

Physiology in taking cognizance of the cell itself and its environment is reduced to its simplest and lowest terms in the cell possessing an individual entity, for a large part of this physiology is found represented in its most rudimentary and elemental forms and consequently quite easily studied.

Nutrition as it is illustrated in *B. subtilis* is easily approached as compared with that of man. It is not difficult to reproduce, change, and control nutritional conditions in a unicellular organism as compared with the multicellular organisms. Methods which enable the investigator to reproduce, manipulate and supervise microörganisms enable him to attack problems excluded from the category of the multicellular physiologist. However, the physiologist of complex forms, as the human, not only has his problems rendered more intricate by the organisms he studies but he also has them multiplied because of the many fold combinations of cells. Bayliss is substantially correct when he says, "The physiology of unicellular organisms, although of considerable importance, is not to be regarded as a general physiology," because the physiology of microörganisms leads only to the point where one phase rule must give way to another phase rule—the physiology, in fact, of any restricted number of organisms harmoniously related can never cover the field of general physiology. Let us examine this more particularly for the purpose of securing the most effective attitude in the study of microbial physiology.

The differences which separate unicellular physiology from the specific human physiology are worthy of consideration. Neither the one nor the other can be considered general or comparative physiology but both have values which are of interchangeable advantage. The unicellular organism enters upon its nutritional career as a free cell found in an environ of food which, in its specific manner, it must prepare for absorption and assimilation. The human first brings its food into a canal, a tube, the alimentary canal, lined with specialized cells which contribute to the preparation, the absorption and assimilation of the food. Enzymes are secreted by the unicellular form into the food medium undergoing preparation for absorption, just as enzymes are secreted by the cells of the walls of the stomach and intestines of the human species. The same purpose is apparent in each case. The food is absorbed through the cell-wall or directly into the protoplasm in the unicellular organism, while in man the cells lining the alimentary tract operate in much the same manner. In the human the distribution takes place by means of a carrier system, the circulation, in order to reach the distant points while with the single-celled organisms the process is one of diffusion. Enzymes are present in both organisms engaged in the process of converting food material into protoplasm and the production of waste. Aside from the distributive method, the nutritive processes are much the same in both. It now remains to conclude which is the more simple to study, the more accessible for study, and the more adaptable to study. In this the single-celled organism has many advantages. This does not constitute, however, general or comparative physiology for either one of these assumes a large number and a varied number of living forms into which creep specific differences.

The similarity paralleled in nutrition could be carried into other functions but this is not the purpose. It is desirable, mainly, to emphasize the possible extension of the simple and even rudimentary basic facts to the field of general physiology.

The complicated structures of the metazoa and metaphytes can scarcely be compared with microörganisms. It is difficult to speculate intelligently on the development of shape and structures in biological forms in the light of present knowledge, yet proceeding from simple forms to the more complex, there is constantly confronting the mind the possibility on the part of nature to adjust form and structure to the growing and expanding demands of protoplasm. The struggle on the part of a microörganism to secure oxygen or to get away from it, the action of light and darkness on the growth of molds and other factors signify as much to a simple single-celled organism as the prehensile tendencies of an insect or an ape. It is something sought by the use of different structures and of different agents. There are so many indications of incipient developments in the unicellular organism that much time and space could be given to speculative possibilities. Only a suggestion is required, however, to start the thinking student to fruitful reflection. The morphologist approaches the subject of the place of microörganisms in nature through the channels of "degenerated forms" from "higher forms" or "types of simple forms in process of evolution." This view does not harmonize with the physiological approach in which functioning supercedes form unless degeneration and evolution is made a matter of functioning instead of form.

A cell as a free unit and a cell as a unit but tied into a community of cells awaken interest. The free cell might be likened to primitive man, a Jack-of-all-trades, but a cell interwoven into a multicellular organism is that of a Jack-of-all-trades, an individual, and a man in a social organization, a specialist. This seems to be apparently true except where the cells simply form an aggregation or a colony in which all cells function alike. There is, too, the tendency of the single free cell to specialize as those of the alcoholic group, the lactic group and many others. To say that they are in the process of functioning together in an organism as the yeast and acetic organisms is wholly chimerical yet very suggestive in nature's slow evolutions. Few facts can be brought forward to disentangle definitely such conceptions.

As a working basis to-day it is necessary, because of restricted human advancement, to consider the cell as the unit of life although its compositional structure when understood may reveal the unit of life for to-morrow.

THE MECHANISM OF CELLS

In the early stages of this book Guilliermond has revealed the structures of microbial cells and has indicated in his treatment the probable relations of these structures to functioning. The immediately foregoing section carries the suggested creative powers of the various cell-structures to molecular foci which seem to be the centers in which vital changes are occurring. It now remains to set forth the mechanism of the cell, functioning as a whole, which can be done only by approaching it through the channels of its activities.

A living organism is conspicuous as a mechanism because it has the power of self-maintenance in the presence of a suitable environment, it can reproduce its like when conditions are favorable, it frequently has the freedom of motion, and possesses many reacting responses to stimuli or expressions of dynamic existence. These are characteristics which belong peculiarly to cell-life.

With food at hand and other favorable conditions the cell becomes an automaton. The material needed in growth and the energy required for operation are obtained without assistance and regulated to meet the exact demands of the cell. If food is plentiful, growth and reproduction reach out to conserve it; if food is scarce, the cell accommodates itself to its limitations by reduced activity or a resting stage. This great power of adjustability while having boundaries, is as useful to the life of the cell as it is wonderful. Likewise, if solid food alone is available, the food is reduced to a liquid form or changed that it may be incorporated in this bit of protoplasm, the cell. When there, it is transmuted into the living substance or is converted from dead matter to living matter. In performing this work, the food consumed has had to provide the energy as well as the material which rejuvenates the protoplasm, for this constant rejuvenating process in the case of protoplasm seems essential to life as well as to the constant supply of energy to make it possible.

There are agents, or properties, or substances of the cell which make this possible, it is true, and there are structures of the cell which are involved in it, but these are necessarily subordinate to the innate protoplasmic forces which give them birth and which in some manner create and supervise them. These detailed features are the subjects of physiological study.

Unicellular organisms are concerned with what may be called respiration. Oxygen, in its free forms, is needed in most instances for body-processes and carbon dioxide is given out. The amount required varies with different organisms and is not determined by the amount present in the air. Other gases are also used. Nitrogen is taken up by the microörganisms which grow on the roots of legumes; marsh-gas, carbon monoxide, carbon dioxide, hydrogen and other gases are claimed to be utilized. Each species of microörganisms seems to possess greater or less specificity in its gas-relations. No species can extend its influence over all amounts of a single gas or to all gases. From this situation it is a forced conclusion that all cells do not function alike, accordingly must have different mechanisms although the capacity for life appears common to all.

Some organisms emit light or manifest radiant energy, other organisms produce pigments which vary with different species, still others elaborate deadly poisons, called toxins, and other substances of pathological significance. Briefly, microörganisms give rise to many different products whether they take the form of secretions, excretions, energy manifestations, or are referable to action upon environmental media. Enumeration is not the purpose here. These very numerous products indicate the many-sided activities of microbial life. If the substances which enter a mechanical device are the same, the products passing out are the same. In this case, the food substances and the conditions controlling the microörganisms may be identical yet the issuing products are different. It follows, therefore, that the mechanism of cells must be very different, yet as mentioned heretofore the life-mechanism is common and the same so far as determinable.

Many unicellular forms have a cell-wall and when no cell-wall is present there is likely to be a distinctive and denser layer of cytoplasm on the outer zone. Some organisms when placed in a dense salt solution will lose a considerable amount of their water-content and shrink while others will not; in other words, some microörganisms have the power of withstanding dense brine while others do not. Osterhout has repeatedly demonstrated that where two salts are together in a medium acting as a substratum for microörganisms there may exist an antagonistic or favorable action of one of the salts upon the absorption of the other salt by the cell but that cells vary in these responses.

It is a common knowledge that cells have a selective action which differs with different microörganisms. The capacity to utilize certain foods in the presence of others, to acquire certain ingredients, and to leave others behind makes for great variability in the functioning of microbial cells.

Spores of some microörganisms are exceedingly resistant, withstanding over twenty hours of heat at 100°. Spores of other species are destroyed in from one to five minutes at 100° under the same conditions. Wide differences are found among vegetative cells likewise. The molecular stability which embodies the life of the cell against heat must be as variable as the dissolution of chemical substances under the influence of heat. Life then can be associated with many kinds of molecular mechanisms although for each species there is a more or less constancy.

In the process of selecting, digesting and assimilating their food, certain microörganisms develop an unusual amount of heat so much that many microörganisms cannot live in its presence, yet these heat-producers (thermophile microörganisms) are dependent upon this excessive heat for their proper growth and functioning.

So far evidences of varied processes existing in different species and strains have been set forth in the products and energy resulting from growth and development, or, more briefly, in the processes of metabolism. These clearly convey the very noticeable variation which must exist in the mechanisms which are responsible for such differences.

There is another approach which will contribute force to what has already been said concerning the mechanisms of living cells.

Chemists have repeatedly shown that cells differ in their chemical compositions. When chemists begin their destructive laboratory manipulations for the purpose of ascertaining what is present in a cell, they may not be working with the real substances or bodies making the living protoplasm. However, the bodies which they do study and which are fairly constant in the same kind of cells or in the same species are doubtless products which in one form or another enter into the complexity of protoplasm. The chemical substances studied in nervous tissue differ from those of muscles; the chemical substances as products of certain species of microörganisms differ from the products of other species. Some of these substances are definitely known to differ and others, although they cannot be satisfactorily determined, are suspected to differ in different tissues and different species of microörganisms.

A specific and more definite consideration of this subject will appear later. Our purpose here is to point out simply that the material making up the molecular mechanism of the cell must be exceedingly delicate and complex because of its products, and the mechanism of one cell must differ very significantly from that of another also because of its products, which as cell-contents are determinable; and further, the mechanism must be highly responsive because of its ready reactions to various influencing agents. All of these evidences seem to accord with the interpretation advanced in the previous chapter—the cell consists of chemical foci and physical forces harmoniously constructed into an operating mechanism, the protoplasm, which is arranged effectively in a cellular laboratory, the cell.

Literature freely consulted and recommended for extended study:

BAYLISS, The Principles of General Physiology.
CHILD, Individuality in Organisms.
CZAPEK, Chemical Phenomena in Life.
HENDERSON, The Fitness of the Environment.
MOORE, The Origin and Nature of Life.
VAUGHAN, Protein Split Products in Relation to Immunity and Disease.
VERWORN, General Physiology.

CHAPTER II

A STUDY OF PHYSICAL FORCES INVOLVED IN BIOLOGICAL ACTIVITIES*

Introduction

It is becoming more and more evident that besides the chemical components and forces which constitute the mechanism and activities of the cell and which will be treated in the next chapter, there are certain physical forces and conditions as important and very dependently related which are operative. In the physical or chemical approach to physiology the writers do not assume the rôle of being chemists or physicists and they write somewhat hesitatingly and reluctantly upon such matters, yet the stream separating the various branches of science must be spanned. The purpose, as writers, will be, therefore, to bring the elementary gist of the physical laws and phenomena, which bear upon microbial physiology, to the attention of the student for memory-helpfulness and suggestiveness. Should greater knowledge or more extensive reading be desired the student is asked to consult the literature appended at the end of this chapter.

Energy

Energy may be most effectively presented in the general law through which it defines itself and governs applications, and in the specific expressions in applications which provide the detailed background. Energy is work or capacity to perform work. This is found in all living units. Work is going on. Whether this energy is designated as *mechanical*, *thermal*, *electrical*, *chemical* makes no particular differences for the form is only one aspect of it.

Energy may be transformed from thermal into electrical or chemical into thermal; in fact "all forms of energy are convertible. The total energy of any substance or system cannot be altered by the mutual actions of its parts." Furthermore, energy is practically indestructible.

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* Prepared by Charles E. Marshall and Arao Itano.

This is true whether energy exist as *potential* energy in the form of rest or as *kinetic* energy in the active form. "In every modification of a material system, not affected by forces foreign to a system, the sum of its potential and kinetic energies remains constant." This statement represents the great law of Conservation of Energy. To determine the total energy of a body is difficult for it usually contains potential and kinetic energy which may not find expression in measurable units. It follows that "the total intrinsic energy of a body or system of bodies is never known. When bodies mutually react it is only the difference of the energy of each body in two states which is considered. If a body has less energy in its actual than in its standard state the expression for its energy is negative." The functionings of a living organism may well be said to be the energizing powers of such organism. These powers are maintained by the food consumed, part of which enters into the synthesis of new protoplasm or in renewal processes, and part furnishes the energy for the work performed. Whether the energy goes to constructive and reconstructive purposes in the maintenance of the mechanism or whether to the manifest liberation of energy as work, it is desirable to remember that the ramifications of either route are multitudinous, intricate and mostly concealed. The principle of the Conservation of Living Forces states "that the difference of the force-functions (or work) at the beginning and at the end of the motion of a system is equal to the difference of the *vires vivæ* (kinetic energies) at the beginning and the end of the motion." This is readily seen to represent only measurable and definite performance and does not in any sense represent potential possibilities.

If living energy is functional energy or functioning then an understanding of it with any degree of comprehensiveness means that it is to be found in the study of the many functional processes of the body in detail. To this attention is directed.

SOLUTIONS

When any substance is placed in or is associated with another substance in such a manner as to enable both to establish a uniform ionic, molecular and particulate mixture, such a combination is called a *solution*. In a *true solution*, this combination, in which the ionic or molecular components are intimately merged or blended and are uniformly distributed or dispersed, is considered a *homogeneous system*. It is also a *one-phase system* because its components are not mechanically separable or physically different. On the other hand, *colloidal solutions* (see colloids and crystalloids) consisting of components which are physically different and mechanically separable form a *heterogeneous system*. Such a system or solution is therefore either *diphasic* or *polyphasic*.

A true solution, composed of two substances, may have one substance regarded as that dissolved and the other as the dissolving agent. If table salt is dissolved in water, the salt would be regarded as the substance dissolved in water as the dissolving agent. In this case, salt would be the *solute* and water the *solvent*. However, just the converse may be equally true: water dissolved in salt. Colloidal solutions are somewhat different because they consist of fine or very small particles in suspension or emulsion. In such solutions, therefore, the suspended particles in solution (called *suspensoids*) or emulsified particles in solution (called *emulsoids*) are the substances distributed or dispersed in a medium or menstruum. The particles are called the *disperse phase* and the medium or menstruum the *dispersion means*. Together they constitute a heterogeneous system, *dispersoids*, also a polyphasic system. Some colloids approximate very closely ionic and molecular solution.

A diagram (Fig. 100) will aid in understanding.

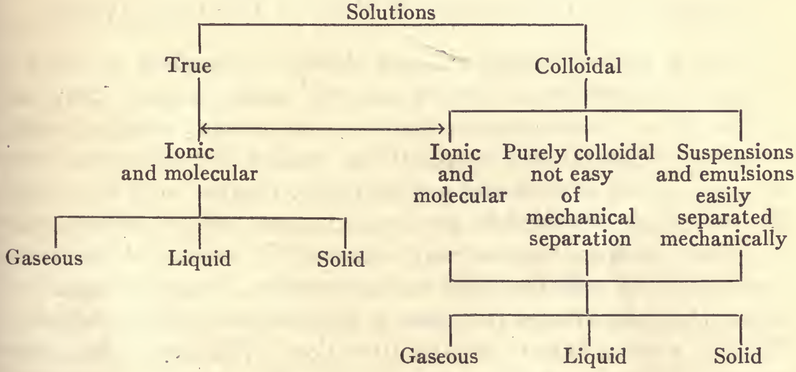
Solutions
True
Colloidal
Ionic and molecular
Gaseous
Liquid
Solid
Ionic and molecular
Purely colloidal not easy of mechanical separation
Suspensions and emulsions easily separated mechanically
Gaseous
Liquid
Solid

FIG. 100.

From the diagram it will also be noted that, whether in true solution or colloidal solution, solutions are not confined to the action of water on some salt but extend in like manner to gaseous, liquid and solid alike, as gas in gas, solid in gas, gas in solid, liquid in gas, liquid in solid, etc.

The solute may exist in the form of a substance which becomes electrically dissociated into its ions, as sodium chloride is dissociated into its ions, sodium and chlorine, in water, when it is called an *electrolyte.* The solute may be resolved only into its molecules in water, as sugar, when it is called a *non-electrolyte.* Such substances are designated usually as *crystalloids*, but this is not uniformly so. Again, instead of using the term solute, in its place there is used the term disperse phase. This is made of small particles greater in size, as a rule, than molecules, and only in suspension. The solution takes on the differentiated form as represented by heterogeneous, polyphasic, and colloidal characters. The so-called solid solution signifies that one solid substance may become distributed throughout another solid substance as in the case of "crystals which are uniformly composed by two crystalline substances which present similarity in crystalline form as well as of chemical composition" or as coloring matter in mineral salts.

The significance of the possibilities of solutions should be grasped to understand the changes taking place in protoplasm through metabolism, egestion and ingestion.

ELECTRICAL CONDUCTIVITY, IONIZATION AND DISSOCIATION

When a salt as sodium chloride (NaCl) is dissolved in water it undergoes dissociation or breaks up into *atoms*—sodium (Na) and chlorine (Cl). Each atom is made up of *electrons* some of which constitute the center and are positively charged with electricity while some are on the outside and are negatively charged with electricity. These latter are more mobile, are not held so tenaciously, and are called the *valence electrons* because they establish the valency of the atom. These electrons could be regarded as the units of electric charge. If sodium (Na) and chlorine (Cl) unite in the formation of sodium chloride (NaCl) it appears to be by electric attraction. This may be due to the transfer of valence electrons from one to the other or attributable to the rearrangement of valence electrons within the atom thus creating the attraction. The *two atoms*, however, remain electrically neutral. *Any free atom or combination of atoms which carries a charge of electricity is called an ion.* When sodium chloride (NaCl) is resolved into the two atoms sodium (Na) and chlorine (Cl) in solution and each contains an electric charge, the process is called *ionization* or *electric dissociation.* The sodium chloride (NaCl) or like substance is called an *electrolyte.* The positive ions are known as *cations* and the negative as *anions.*

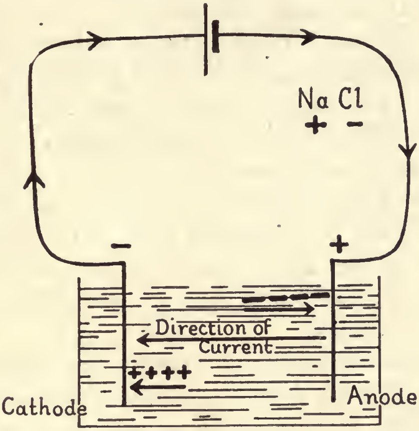

Fig. 101.—Movement of Electric Current and Ionization.

In the dissociation of sodium chloride (NaCl) in water a negative unit of electricity in the form of a valence electron goes with the chlorine (Cl) and a positive unit of electricity in the form of a valence electron is thus established with the sodium (Na). These electrons are further attached to molecules of water together with which are formed the electrically charged ions. The loss of the negative valence electron by the sodium (Na) when it passes with the chlorine atom (Cl) leaves the sodium atom (Na) positive or, in other words, the withdrawal of the negative electron from the sodium (Na) disestablishes the neutrality of the sodium (Na) by making it positive and that of the chlorine (Cl) by making it negative. The result creates a flow of electricity because there is a difference in the *potential* of the two atoms. The potential determines the flow of electricity in much the same manner as pressure determines the flow of a liquid.

Such resistance as is offered by a solution to a current of electricity may be measured by the Wheatstone Bridge.

When an electric current is introduced into a solution of sodium chloride (NaCl), the ions carry the electricity, sodium (Na) carrying the positive charge and chlorine (Cl) the negative charge. The positive or sodium ions gather at the electrode or pole or *cathode*, the pole by which the current leaves the solution; the chlorine ions which have the negative charge pass against the current to the *anode*, the pole by which the current enters the solution. Those gathered at the cathode are the *cations*, those at the anode, the *anions*.

The electric relations existing between sodium (Na) and chlorine (Cl) in sodium chloride (NaCl) also exist in hydrochloric acid (HCl) in which hydrogen and chlorine bear the same relationships as sodium (Na) and chlorine (Cl) in sodium chloride (NaCl).

The strength of the acid is measured by *displaceable hydrogen atoms*. In nitric acid (HNO_3) one hydrogen atom (H) is displaceable, in acetic acid (CH_3COOH) only one hydrogen atom (H) is displaceable, for the hydrogen atoms of the methyl group (CH_3) are not displaceable.

If an acid is neutralized it is the hydrogen which has been substituted by some other cation.

Since it is the hydrogen (H) which determines the strength of an acid and this hydrogen (H) has definite values in every acid then it must be possible to establish a standard of measurement by taking a gram-molecular solution as of hydrochloric acid (HCl) which is called a normal (N) solution. On the other hand, alkali solutions may be established of standard values against the acid standards. In these the hydroxyl (OH) ions act as the neutralizing agents against hydrogen (H) ions of the acids in such proportion as to form water.

"True Reaction" (*"True Acidity," "True Alkalinity," "True Neutrality"*).*—The "true acidity" of an acid solution is brought about by the dissociated (hydrogen) ions; therefore the acidity is proportional to the concentration of the dissociated hydrogen ions, and not to the total gram molecules of acid present. For example, if one-tenth normal hydrochloric acid is taken, approximately only 91 per cent. of the total amount of acid becomes dissociated. The "true acidity," *i.e.*, the hydrogen ion concentration, of this solution is only 91 per cent. of the one-tenth normal hydrochloric acid, or ninety-one thousandths normal. The dissociation of weak acid is still less. For instance, in a solution of

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* Bull. 167. Mass. Agr. Exp. Sta. by Arao Itano.

one-tenth normal acetic acid only 1.3 per cent. approximately of the total acid is dissociated, and the hydrogen ion concentration of this solution is therefore thirteen ten-thousandths normal. The "true acidity" of one-tenth normal hydrochloric acid is also about seventy times greater than that of one-tenth normal acetic acid, although both solutions contain the same amount of acid.

The same holds true with the electrically dissociated base in which the metallic and hydroxyl ions are dissociated. The "true alkalinity" of such a solution is not determined by the total amount of base present, but exclusively by the concentration of dissociated hydroxyl ions. For example, in a one-tenth normal solution of the strong base, sodium hydroxide, about 84 per cent. of the total amount of the base is dissociated, and in the case of a weak base, such as ammonium hydroxide, approximately 1.4 per cent. of the total amount of the base. The "true alkalinity" of these solutions, therefore, is eighty-four thousandths normal and fourteen thousandths normal, respectively. Thus, regarding the alkalinity as in the case of acidity, we may say in conclusion that "true alkalinity" of a solution is proportional to the concentration of hydroxyl ions.

From the above discussion, "true neutrality" of a solution may be stated as follows: it is a solution in which the same amount of H and OH ions are present. For example, a "true neutral solution," viz., pure water, contains as many hydrogen ions as hydroxyl ions. It can be expressed as follows:—

$$H_2O \leftrightarrows \overset{+}{H} + \overset{-}{OH}$$

in which $C_{\overset{+}{H}} = C_{\overset{-}{OH}}$, C indicating the concentration.

Again, a solution may not necessarily be neutral, although it contains equivalent quantities of acid and alkali. For example, if a solution which contains hydrochloric acid and sodium hydroxide is taken, it can be expressed in the following manner:—

$$\underset{\substack{\text{hydrochloric}\\\text{acid}}}{\underline{\overset{+}{H}\quad\overset{-}{Cl}}} + \underset{\text{sodium hydroxide}}{\underline{\overset{+}{Na}\qquad\overset{-}{OH}}} = \underset{\text{salt}}{\underline{\overset{+}{Na}\,\overset{-}{Cl}}} + \underset{\text{water}}{\underline{HOH}}$$

This solution is neutral only when it contains just as many hydrogen as hydroxyl ions, or when both the acid and alkali are equally dissociated.

It is understood, therefore, that the "true acidity, alkalinity and neutrality" are not determined by the amount of such substances present, but entirely by the H and OH ion concentration.

Theory of H Ion Concentration.—The announcement of the theory of electric dissociation by Svante Arrhenius, in 1887, marked a new era in physical chemistry. It was F. Kohlrausch and A. Heydweiller who demonstrated that even the purest water is a conductor of electricity, and accordingly prepared a distilled water of the least specific conductance. They measured the specific conductance by means of electric conductivity. Later, other methods for the estimation of dissociation were established, and the results obtained by Kohlrausch were confirmed. Now it is proved that a very small portion of the water molecule is dissociated into two electrically charged parts (or ions), as follows:

$$H_2O \leftrightarrows \overset{+}{H} + \overset{-}{OH}$$

Its dissociation takes place according to the law of mass action in accordance with the following equation:—

$$\frac{(H)(OH)}{(H_2O)} = K \qquad (1)$$

in which K denotes the ionization constant; that is to say, the product of the hydrogen and hydroxyl ion concentration, divided by the concentration of the undissociated water molecule, should be constant.

The concentration of water is generally constant. Therefore it may be expressed as follows:—

$$(H).(OH) = Kw \qquad (2)$$

in which Kw denoted $K.H_2O$, or ionization constant of water.

Equation (2) is another form of equation (1).

This ionization constant of water has been determined by several noted physical chemists, and found to be 10^{-14} at 22°; that is,

NOTE.—(H) and (OH) express the concentration.

$$(H).(OH) = Kw \quad \text{or}$$
$$Kw = 10^{-14} \qquad (3)$$

Since pure water is a neutral solution it contains the same number of dissociated hydrogen and hydroxyl ions. Therefore equation (3) can be expressed as follows:—

$$10^{-7} \times 10^{-7} = 10^{-14} \qquad (4)$$

That is, a pure water contains of each 10^{-7} dissociated hydrogen and hydroxyl ions, or .0000001 gram ions per litre, which is, in a general term, one ten-millionth normal $\frac{N}{10,000,000}$. The acidity, alkalinity and neutrality, therefore, are expressed in terms of hydrogen ion concentration in the following manner:—

Acid reaction $(H) > 10^{-7}$
Alkaline reaction $(H) < 10^{-7}$
Neutral reaction $(H) = 10^{-7}$

That is, in an acid solution there are more than $\frac{1}{10,000,000}$ gram molecule of dissociated hydrogen; in an alkaline solution, less; and in a neutral solution, just $\frac{1}{10,000,000}$ gram molecule. Thus the reaction is usually expressed in terms of hydrogen ion concentration unless it is indicated otherwise.

From the above discussions it is readily seen that if the ionization constant is known, and the hydrogen ion concentration is determined experimentally, then the hydroxyl ion concentration can be calculated. The determination of hydrogen ion concentration is accomplished by the use of the gas cell, of which the principle is based upon the potential of the chain. This chain as described in physical chemistry, consists of—

Hg—HgCl	n/10KCl	conc. KCl	solution	Pt H_2
calomel	electrode	concentrate potassium chloride in a dish.	(unknown)	platinum electrode saturated with hydrogen gas.

The potential of such a chain can be determined by the usual physical method. Then the relation between the measurement of potential and hydrogen ion concentration can be calculated by the following equation:—

$$P_{\dot{H}} = \frac{P - 0.3377}{0.0577 + 0.0002\,(t^\circ - 18^\circ)}$$

NOTE.—(X) = notation of the concentration of ions.

where—

P_H—the term adopted by S. P. L. Sörensen to express the exponent of gm.- equivalent of hydrogen ions per liter.

P—the total E. M. F. of the chain. It can be determined by the following equation, having the apparatus arranged as it is shown in the diagram:—

$P = \frac{R_1 \times 1.0189}{R}$ in which R_1—the bridge reading for the chain against an accumulator.

R—the bridge reading for the accumulator against the normal element.

1.0189—the voltage of the normal element at 18° (standard).

0.3377—the sum of potential of calomel electrode (N/10 KCl) and hydrogen electrode in a solution where the hydrogen concentration is normal (H) = 1 or $P_H = 0$.

0.0577—thermodynamical factor at 18° which is influenced by temperature, 0.0002 for each degree centigrade, or it changes as follows:—

$0.0577 + 0.0002\,(t° - 18°)$, of which t° equals temperature at the time of determination.

After $P_{\dot{H}}$ is determined it is necessary to understand the value of H-ion concentration, although the experimental results are generally expressed in $P_{\dot{H}}$. It will be shown at the end of an example, illustrating the application of the formula as well.

Example.

$t° = 19.2°C$ (constant during the experiment).

$R_1 = 307.0$ (constant reading on the bridge at five minute interval).

$R = 500.2$ (as above).

E. M. F. of the normal element = 1.0189.

Then the total E. M. F. of the chain can be calculated as follows:—

N.E. = normal element.
Ac. = accumulator.
x = the chain.
1000 = scale on bridge.

$$\frac{307.0}{1000} = \frac{x}{Ac.}; \quad \frac{500.2}{1000} = \frac{N.E.}{Ac.}$$

$$x = \frac{307.0\ Ac.}{1000} \qquad (1)$$

$$\frac{500.2}{1000} = \frac{N.E.}{Ac.}$$

$$Ac. = \frac{1000\ N.E.}{500.2}$$

$$= \frac{1000 \times 1.0189}{500.2} \qquad (2)$$

Substituting (2) in (1),

$$x = \frac{307.0}{1000} \times \frac{1000 \times 1.0189}{500.2}$$

$$= 0.6254 \text{ volt, which is expressed p.}$$

Substituting the value for p in the formula,

$$P_{\dot{H}} = \frac{0.625 - 0.3377}{0.0577 + 0.0002\ (19.2 - 18)}$$

$$= 4.967$$

or in terms of H ion concentration,

$$P_{\dot{H}} = 4.967 = -4.967(\log.\ H)$$

$$10^{-4.967} = 1.0789 \times 10^{-5}$$

$$H = 0.000010789$$

Besides the apparatus listed below, a H-generator was employed, which is a good-sized Kipp's generator used with a series of washing bottles and drying tube, consisting of (*a*) 30 per cent. KOH, (*b*) alkaline pyrogallic acid, (*c*) conc. H_2SO_4 and soda lime in U-tube. Since a considerable amount of CO_2 is produced during the course of metabolism, the same precaution is taken as with blood. For this purpose Hasselbach's electrode with shaking arrangement is employed.

In setting up the apparatus special attention should be paid to rigidness, insulation and temperature. In order to meet with these requisites the apparatus was placed on a big central table in the laboratory. First, one dozen large glass rings of the same height were distributed over the top of the table. These supported a thick glass plate on which several blocks of paraffine for each piece of apparatus were placed. Thus it was possible to obtain a perfect insulation.

In preparing the different parts of the apparatus extreme care should be exercised to obtain an accurate result. The method for the preparation of the normal element, calomel electrode, gas cell, and also calibration of the bridge wire, etc., is described in detail in Findlay's "Practical Physical Chemistry." Every contact should be carefully made, so that accurate readings can be obtained. It is worthy of mention that the diffusion potential between n/10 KCl calomel electrode and the solution to be tested is reduced by interposing the saturated solution of KCl as it is indicated by K on the diagram. For the standardization of the electrode it was first platinized with general precaution; then the hydrogen ion concentration of the mixed solution (7 c.c. of m/15 KH_2PO_4, 3 c.c. of

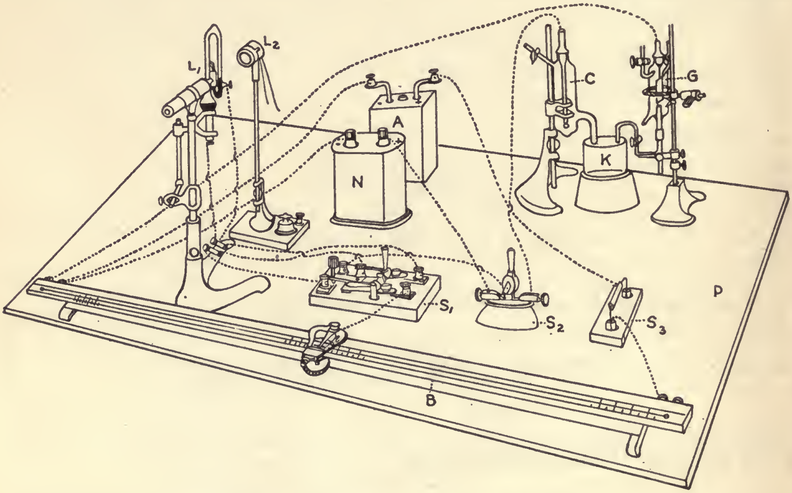

Fig. 102.—Apparatus employed in determination of H-ion concentration.

Description of Diagram

L_1—Lippmann's capillarimeter.
L_2—Tungsten lamp.
A—Accumulator.
N—Western normal element.
S_1—Switch with quick short circuiting key.
S_2—Three-way switch.
S_3—Two-way switch.
C—Calomel electrode.
K—Concentrated KCl cup.
G—Gas cell.
B—Bridge.
P—Thick glass plate.

m/15 Na_2HPO_4) was determined at different intervals. After the readings became constant there was a difference of 0.0005 volts between the theoretical data and the results obtained.

With the above facts in mind it becomes possible to enter upon a more intelligent discussion of the methods involved. It has been stated previously that most microbiological experiments, having for their purpose the study of reaction upon microbial life, fall under the following procedures:—

(*a*) Kisch's method.

(*b*) Ordinary titration method.

(*c*) Colorimetric method.

It is well known that Kisch's method is a dilution method wherein a certain number of gram molecules of an acid or alkali are diluted to a definite quantity for the purpose of ascertaining the influence of the reaction upon the life of bacteria. There are two distinct ways to apply Kisch's method, namely: (*a*) immersing the bacteria in different dilutions of acids or alkalis in pure water for different periods of time by means of silk threads or any other convenient agents, and then testing their vitality; or (*b*) adding a known percentage of acids or alkalis directly to the culture medium (usually solution). In either case the results obtained by Kisch's method indicate neither the influence of "true reaction" upon microbial life nor the influence of molecular concentration, because, as Lingelheim has shown, different acids of the same molecular concentration have varying influence upon microörganisms, and the degree of influence is parallel to the dissociation constant of an acid or alkali. This is especially true in the case of the second manner of application, (*b*), where adsorption is caused by the culture medium.

The ordinary titration method is generally employed in adjusting reaction of culture medium, and also to measure the amount of acid or alkali produced in the course of physiological tests. This method is inaccurate in the study of physiological liquids containing more or less amphoteric substances and a comparatively small quantity of H or OH ions. In other words, it is impossible to determine the "true reaction" in such a liquid by this method. Fuller's and Schültz's methods of adjusting the scale of reaction of culture media are scientifically condemned by the recent investigation of Clark, who showed the fallacies of the titrimetric method. Again, the adsorption phenomenon caused by the amphoteric substance in the course of titration is well known, and, in the case of albumin, is usually expressed in the following manner:—

$$\text{In acid solution}\quad \text{H. albumin. OH} = \overset{+}{\text{H albumin}} + \overset{-}{\text{OH}}$$

$$\text{In alkali solution}\ \text{H. albumin. OH} = \overset{-}{\text{Albumin OH}} + \overset{+}{\text{H}}$$

The correctness of the above statement has been experimentally demonstrated by Sörensen, Clark and others.

In many cases the colorimetric method gives fairly accurate results, but it has been noted that the presence of neutral salts as well as amphoteric substances interfere with the determination. It may, however, be employed successfully if it is standardized for the particular liquid. Lately Clark and Lubs employed the principle of the colorimetric method for the differentiation of the colon-ærogenes family, using suitable indicators. They have based their experiment upon the wide divergence of the hydrogen ion concentration in a culture of one group and of the other, and distinguished this difference by means of paranitrophenol or methyl red. The use of this method for physiologic work other than for microbiology has been practiced by many. Sörensen and Palitzsch determined the hydrogen ion concentration of sea water. Henderson and Palmer used it in determining the acidity of urine to diagnose normal and abnormal conditions. In any case, the colorimetric method should be standardized previous to its use, by means of the hydrogen electrode.

Examining these methods critically in the light of physical chemistry they are not satisfactory for the purpose of ascertaining the influence of the so-called "true-reaction" upon microbial life. The hydrogen electrode was devised to determine the hydrogen ion concentration, and it has been used successfully in biologic fields.

Surface Tension

Due to such forces as cohesion and adhesion the particles of bodies have a tendency to come together in the same manner as bodies fall to the earth. This property appears to lie within the molecular forces of the body and seems to have a circumscribed and limited area of action. If a center is assumed in the form of a molecule, this area over which an influence of attraction is exerted would be in the form of a sphere and would be recognized as the *sphere of molecular action.*

The layer of a liquid representing its surface plane with a depth equal to the radius of the sphere of molecular action would be the *surface film.* If a particle lies within or inside of this surface film it follows that with this particle as a center, the radius of its sphere of activity will extend beyond and above the surface film, but if this particle lies without and below this surface film the molecular forces on all sides will be equal and an equilibrium established.

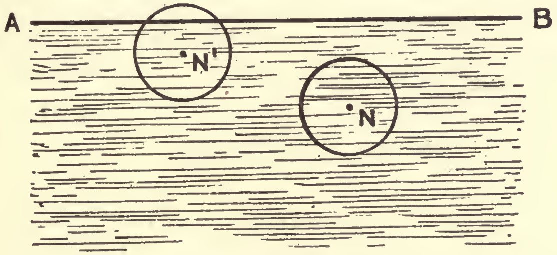

FIG. 103.—Illustrating surface forces.

This is illustrated in Fig. 103. *AB* is the plane surface of a liquid. *N* is a particle with its circumference indicated in which all forces are equalized. *N'* is a particle in which the forces downward are greater than the forces upward. The forces lying above the plane surface of the liquid *AB* appear to be less than the forces operating immediately below the plane surface *AB* in the liquid, yielding a considerable increase of pressure in the liquid. This increased pressure is known as the *molecular pressure* of the liquid.

The surface film described above possesses a pull or is under tension or is the *surface tension* of the liquid. If an iron ring has stretched across its interior surface a soap film and a silk-thread loop is carefully rested upon it and run to the iron ring, the film inside the silk loop may be broken readily by any penetrating substance when the sides of the loop will spread out in the fullest degree drawn by the soap film without. Much like this is the floating of a rubber band on water. If a rod dipped into alcohol is touched to the surface of the water within the band the water film without pulls the band into its full circular form (Fig. 104*b*) through the reduction of the surface tension of the water within by the addition of alcohol. This pull of the water without may be broken by the addition of a trace of alcohol. In this case the rubber band again resumes its former shape (Fig. 104*a*).

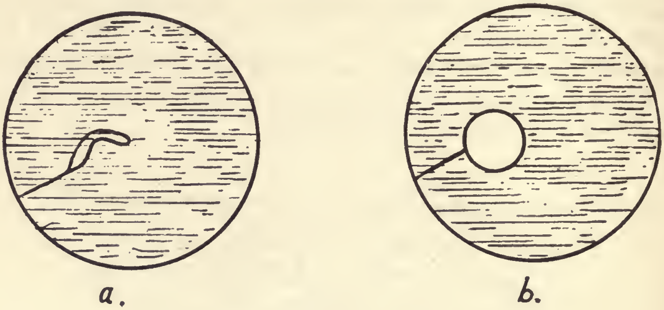

FIG. 104.—Illustrating surface pull.

In the case of an oil drop on water the oil runs to a ball because of the cohesive forces within the oil and the lack of sufficient gravitational and molecular forces or pulling forces within the water film. Mercury for the same reason distributes itself in many small globules when split. On the other hand if the forces below or upward attraction has a stronger pull than the cohesive forces, then the oil would spread out as on a clean glass.

The definite reactions resulting from experiments as employed in demonstrations of the above nature at once establish the possibility of accurate quantitative measurements. It has been found that substances vary very materially in their surface tensions. Kimball* gives the following table:—

SURFACE TENSIONS IN DYNES PER CENTIMETER

	Air	Water	Mercury
Water	73.5	.....	412
Mercury	539.0	412	...
Olive oil	34.3	20.6	335
Alcohol	24.5	.....	...
Ether	17.6	.....	...

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* "College Physics." For Method of Measurement, also consult Kimball.

The possible effect surface tension may have upon the outer layer of protoplasm constituting a cell and in the formation of a membrane, its relation to nutritional functioning and in cellular movements, its suggestiveness in connection with form and its probable importance with alterations of various kinds render it a topic of prime importance although its values are very much dimmed by incomplete knowledge.

ADSORPTION

Spongy platinum has the power to take up considerable quantities of hydrogen gas and also oxygen gas into its mass; charcoal takes coloring material from solutions; it also takes up gases; platinum black takes up acetic acid; calcium carbonate takes up sodium nitrate. When substances are so taken they are said to be *adsorbed.* This power seems to be resident in the adhesive forces of the extensive surfaces which exist through the multiplicity of particles in the substance as in charcoal. It has been defined as the local concentration or condensation of dissolved substances at the interface between two phases. For instance, the interface existing between the dispersoid phase and dispersion means intensifies the surface action to such an extent that there is a concentration, a condensation. Reactions are apparently accelerated. The contact of hydrogen and oxygen in spongy platinum produces water. The action many times is that of catalysis as the oxidation of alcohol to acetic acid by platinum black. The adsorbing substance does not seem to enter into the chemical reaction which may occur but may be recovered intact.

These reactions are influenced by temperature, pressure, electric forces and nature of the substance.

By this phenomenon of nature soluble salts are held back in soils and not washed away by rains. The action of certain disinfectants is explained by the deposition or concentration on the surfaces of micro-örganisms; the reaction of toxin with antitoxin simulates adsorption phenomena more closely than mass action; the sensitization of bacteria by opsonins and the ingestion by leucocytes also resemble adsorption acts; the peculiar reactions of enzymes are regarded as similar to adsorption; the formation of a membrane upon exposed protoplasm in the case of a crushed protozoön also appears to be the result of the adsorptive action of certain substances.

BROWNIAN MOTION

This phenomenon is familiar to students of microbiology. When studying some bacteria in a hanging-drop under one-twelfth oil immersion objective, this movement may be seen. It is not only visible with some of these living organisms but extends to many substances existing in very fine particles and suspended in certain media. It is a common phenomenon among colloidal solutions.

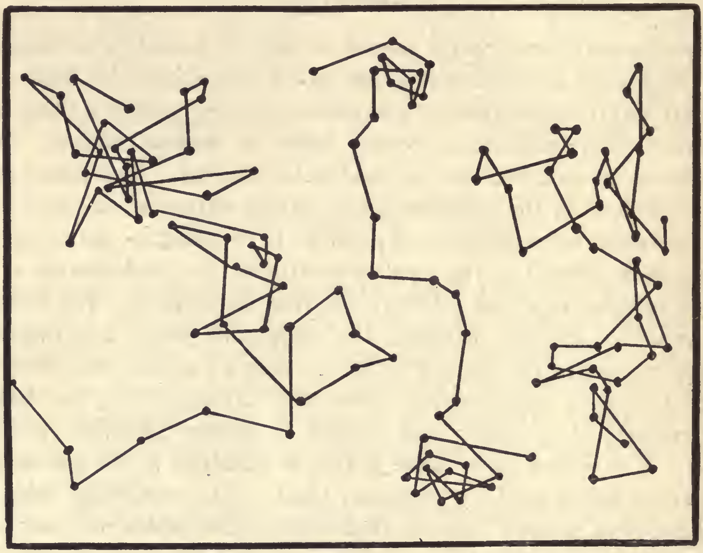

FIG. 105.—Illustrating Brownian movement (*After Perrin*).

The character of the movement is well illustrated by Perrin* (Fig. 105) who has made a special study of the subject. The path is a straight line until opposed when it rebounds in another straight line producing a zig-zag route.

The cause of the motion appears to be inherent in the molecular movements of the dispersion means of a colloid, of the liquid in which the particles are suspended. The direction of the particles as stated above, is that of a straight line until a collision with the invisible molecules takes place when the rebound sends the particles in a straight line in another direction. This process continues indefinitely. The particle subject to these molecular movements and forces responds on the whole as a football might be knocked indiscriminately about a field by a group of unorganized school-boys.

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* **Perrin, M. Jean, Brownian Movement and Molecular Reality.**

Such movements of colloidal particles are supposed to render colloidal solutions more stable. This taken together with the density of the dispersion means, its viscosity, the size of the particles in which surface action becomes more evident, and the electric charge probably accounts in large part for the permanency of the dispersoid state. The velocity of the movement of particles depends upon many of the factors associated with colloidal permanency. An increase of temperature quickens the movement not through convection currents but by the molecular activity; viscosity acts in a seeming frictional capacity; the density acts as if there was a tendency to close in on the particles with forces which are made effective through the multiplicity of molecules; size apparently is much like keeping a small ball in the air as compared with a large ball.

Again, the size of particles which are subject to exact measurement is related to the rapidity of their movement. Exner has made this comparison:—

Diameter of particle in μ	Velocity of particle in μ per second
1.3	2.7
0.9	3.3
0.4	3.8

It will be seen that the smaller they are the more rapidly they move.

Brownian motion, because of the forceful drive furnished by the molecules, appears to be an important factor in diffusion and osmotic bearings.

Diffusion, Osmosis, Dialysis, Permeability

If a twelve per cent. warm gelatin solution is brought in contact with water of the same temperature, currents, not convection currents, are seen radiating, spreading and extending from the gelatin solution into the water until finally they merge with the water and are lost to sight, when the entire mass becomes uniform and homogeneous. A strong salt solution, when placed in the bottom of a cylinder and water carefully poured above it, will little by little work up into the water until the whole is one homogeneous concentration. This would also be true if the water in the former case were substituted by a weaker solution of gelatin or, in the latter case, by a weaker solution of salt. There is a tendency to equalize or become uniform and homogeneous.

Microbiologists are also familiar with certain special phenomena. Litmus agar becomes reduced by the growth of microörganisms. Oxygen has been consumed. When the culture is allowed to remain exposed to the air for a time, the microörganisms cease to grow and multiply; the litmus, beginning at the top, gradually resumes its color as the air works its way down through the culture. There has been a gradual diffusion of the air throughout the litmus agar. Many cultural phenomena could be recalled in this connection. One will suffice. The heating of culture media to drive off the air for anærobic cultivation is of frequent occurrence, for it is well known how the air soon penetrates when media are allowed to stand.

Apparently there are encountered in the first two paragraphs distinct phenomena or a single phenomenon modified in the one or the other instance. The usual explanation, however, is covered by the word "*diffusion.*"

The recent developments in the understanding of diffusion attribute to diffusion the same forces operating in gases. It is the *drive* possessed by the molecules to expand or press out until equalization or equilibrium is established. This movement is from the more concentrated solution toward the less concentrated or toward the pure solvent. The nature of a substance, difference in concentration and temperature materially influence this movement.

This accords with the forces of *osmosis* as well: The pressure upon the obstructing membrane through which the particles, molecules or ions of a substance are attempting to make their way is called *osmotic pressure;* the particles are held back or restrained in their movements outward. It has been found, however, that "the osmotic pressure of a dissolved substance is exactly the same as the gas-pressure which would be exerted if the solvent were removed and the dissolved substance in gaseous form were left behind to occupy the same volume at the same temperature." It is also known that "where two liquids which will mix are separated only by a porous membrane there is a movement of the liquid in both directions through the membrane. The greater movement is usually from the less dense to the more dense so as to cause the line of the more dense liquid to rise above that of the less dense. This action increases with the temperature and is proportional to the concentration of the solution. In the illustration of diffusion above by means of gelatin and salt, diffusion follows its natural course; but in the case of oxygen penetrating litmus agar or any other medium the action may be regarded as modified diffusion or osmosis in which the medium acts as a barrier to the medium-content but allows the gas (air) in its drive onward to pass and diffuse throughout. This leads to the significance of *permeability* of membranes.

Much attention has been given to the study of membranes as they relate so closely to the membranes of cells which are concerned with living processes. It is more or less simple to demonstrate the passage of water and the restraining of a substance like sugar by means of parchment paper. This is a common experiment. In a thistle tube with its mouth covered with parchment paper place a sugar solution to the neck. When plunged into water, the water will pass in and appear in the rising line. At the same time no sugar passes through and out into the water. The molecules appear too large to pass through the pores. This membrane is *semi-permeable* since it permits water to pass but restrains sugar. A membrane or anything which does not allow anything to pass, as glass, would be called *impermeable.*

Whether *dialysis* (passage through a membrane in the separation of colloids and crystalloids) or the permeability of membranes is traceable to its sieve-like nature, its chemical reaction, or to its solvent action or to more than one of these is a mooted problem of prime interest but out of place in this consideration. Some data throwing light on the action of membranes may be helpful, however. The Bechhold ultra-filters made of collodion, which may be graded to varying porosities, have been employed in such a manner as to illustrate the permeability of membranes. Some substances will pass while others will not until the size of pores are adjusted. The membrane resulting by the contact of potassium ferrocyanide with copper sulphate allows water and potassium chloride to pass while it withholds potassium sulphate and other salts. In nature membranes may be permeable to certain salts at times and impermeable at other times. Osterhout has demonstrated many of the possibilities of protoplasmic permeability. Speaking in very general terms, permeability as manifested in living cells and measured by electric conductivity, as has been the case with Osterhout's investigations, may be decreased in its reaction to sodium chloride by alkaloids, as caffein, nicotine and cevadine, by bile salts as sodium taurocholate, and by acids as hydrochloric acid; on the other hand, it is increased by alkalis, by certain isotonic combinations of salts or balanced solutions and by acids following the first stimulation. Protoplasm may vary widely from the normal in its permeability and both vegetable and animal cells respond in much the same general manner.

Although these specific facts may be very limited compared with the entire field of permeability possibilities to which a living organism is exposed, they do, however, indicate that the membrane or protoplasmic protective surfaces have the power to act in a selective manner *per se* or to yield to environing forces or influences which control or make life possible by antagonisms, reactions, neutralizations and other agencies among themselves.*

Osmotic pressure, following the laws of gas pressure, represents the pressure exerted by the particles of a given volume of a solution. The particles, molecules, or ions, of the solution, as in gas are constantly on an outward drive, an expansive drive, and they carry with them much force which is proportional to the concentration of the solution and is subject to the influence of temperature as stated previously. Also the osmotic pressure of a given quantity of substance is inversely proportional to the volume (p. 174). When, therefore, a solution of a great concentration is separated from that of less concentration with a semipermeable membrane between, the pressure exerted on each side of the membrane will be proportional to the concentration of the solutions. The pressure will be influenced by temperature and there will be a stirring of the unequal forces to gain an equilibrium. If only the solvent in the two solutions of different concentration, as just referred to, passes the membrane, then there will be movement toward and a gradual dilution of the more concentrated until it becomes equalized with the other; if both solvent and solute pass there will be by the passage of both through the not truly semipermeable membrane an effort to equalize with more or less exchange from both solutions as in the case of obstructed diffusion.

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* The writers call especial attention to Osterhout's work and that of his students as published in the Journal of General Physiology, Journal of Biological Chemistry, Science and the Botanical Gazette.

In the discussions of osmotic pressure there has been constantly in mind the action of solutions upon microörganisms. Either a cell-wall or membrane exists as a distinct structural part as in the yeast cell or the protoplasm comes in contact with its surrounding medium without any distinctive cell-wall or membrane as in the amœba. Whether there is a layer of protoplasm on the outer surface of the amœba which has the functioning capacities of a distinctive cell-wall may not be easily asserted for there is evidence pointing to the two possibilities. Inasmuch, however, as the passage of materials into the substance of the cell is really that of diffusion or a modification of it, and species and varieties respond differently to this diffusion, it is easily seen that every species at least must be considered by itself in this respect and values likewise determined.

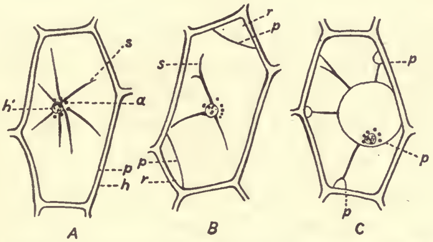

FIG. 106.—Plasmolysis in cells (*After DeVries from Macleod*).

It is well known that water will pass into some cells and cause them to swell or fill out when apparently the substance of the cell or its fluid content is more concentrated than the surrounding medium. On the other hand, when the medium without is more concentrated than the cell-contents, water flows from the cell toward the more concentrated solution outside of the cell and accordingly the cell shrinks. This is many times made evident by the contraction of the protoplasm. This process in which the water is abstracted from the cell through osmotic pressure is known as *plasmolysis*.

COLLOIDS AND CRYSTALLOIDS

Since the time of Thomas Graham who established these two classes of substances there has been a growing interest in them. At present, however, instead of dividing substances into two classes placing one substance in one class, as colloids, and another distinct substance in the second class, as crystalloid, one and the same substance may exist in both classes. Therefore, two conditions or states of the same substance may be found, the one the colloidal, the other the crystalloidal condition or state. Consequently, substances cannot be divided in accordance with the early views of Thomas Graham, but the conditions or state under which they exist, may be so divided into *colloids* and *crystalloids*. The resolution of these classes, as will be seen, is fraught with many difficulties.

The usual ultimate chemical and physical conception of matter is molecular and atomic. Associated with this are physical properties and qualities. Comparatively recently, matter has taken on new interpretations for the molecule and atom have extended to the electron and sub-electron possessing definite electric potentialities. In the opposite direction there appears to be an aggregating or massing power along with the solvent belonging to the molecule in which the atom and electrons may be active. This aggregating power does not seemingly manifest itself in the same manner with all substances; in other words the particle resulting from this aggregation in the case of hydrated silicic acid may not be executed in the same manner as in the case of ferric hydroxide; in the case of gelatin, as in the case of casein; in the case of particulate gold as in the case of particulate carbon. Such aggregate particles, apparently, are different from the molecular or atomic particles in their structures and reactions and the term aggregate does not convey the true structural nature. In molecular reactions chemistry follows its usual course; in the particulate reactions, physical manifestations form the basis of recognition. These differentiations, while helping to distinguish between the well known structures met in crystalloidal chemistry and the more or less amorphous structures of colloidal chemistry cannot be held as a fast cleavage line because they merge into each other and too little is understood of the structure of colloids. They, however, are suggestive, directive and helpful.

Crystalloids form, as a rule, true molecular or ionic solutions (see *Solutions*, p. 156) while colloids form solutions of a more or less mechanical character; the former produce a uniformly dispersed homogeneous system not separable mechanically, the latter give rise to a solution mechanically separable and not uniformly dispersed—a heterogeneous system. Also the former give rise to a one-phase system while the latter yield a polyphasic system. The solution of colloids is concretely illustrated by reference to casein in milk, or gelatin in aqueous solution, which is easily grasped to differ from a solution of salt, a crystalloid, in water. In colloidal solutions the particles are referred to as the *disperse phase*, the medium in which they are found, the *dispersion means* and the solution as a whole, a *dispersoid*. In the event that gold be reduced so fine that its suspension gives rise to a colloidal solution, gold would be the disperse phase, water the dispersion means and the solution or suspension as a whole, the dispersoid. The gold would also represent one phase and the water another phase, resulting in a diphasic heterogeneous system. Where the gold particle and the water meet or at the point the disperse phase and dispersion means come together or are in contact is the so-called *interface* so important in surface energy. Sometimes the disperse phase is called the *internal phase* and the dispersion means the *external* or *continuous phase*.

Dispersoids exist as *suspension-colloids* or *suspensoids* and *emulsion-colloids* or *emulsoids*. The former designate the disperse phase to be a solid and the dispersion means a liquid (*lyophobic colloids*); the latter designate the disperse phase to be a liquid and the dispersion means also a liquid (*lyophilic colloids*). As an example of the former, colloidal gold as the disperse phase and water as the dispersion means is satisfactorily typical; as an example of the latter, gelatin as the disperse phase and water as the dispersion means qualifies, although the gelatin is very close to a solid at times but probably still in a hydrated condition.

This attempt to divide the colloidal condition or state into two classes is quite general. In the above paragraph Von Weimarn* and Ostwald have made the division into suspensoids and emulsoids, Perrin† into lyophobic and lyophilic. Noyes‡ contributes another division: "As types of these I would draw your attention to these aqueous solutions of gelatin and of colloidal arsenious sulphide. The former class possesses a much greater viscosity than that of water; the latter does not appreciably differ from it in this respect. The former gelatinizes upon cooling or upon evaporation, and passes again into solution upon heating or addition of the solvent; the latter does not gelatinize upon cooling, and if gelatinized by other means it does not redissolve upon heating. The former is not coagulated by the addition of salts (unless in excessive amount), the latter immediately gives an abundant precipitate. We have therefore to distinguish the viscous, gelatinizing, colloidal mixtures, not coagulated by salts, from the non-viscous, non-gelatinizing, but readily coagulable mixtures. The former class I shall designate *colloidal solutions*, the latter *colloidal suspensions*." Other divisions of much the same character have been suggested. All lack in fundamental significance. They follow much the same cleavage line but it possesses a ragged fringe. Whether of great or permanent value or not, it is useful until a more definite, basically sound, division can be established.

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* Von Weimarn, Grundzuge der Dispersoid Chemie (Steinkopff, Dresden), 1911.

† Perrin, J., J. Chim. Phys., 3, 50, 1905.

‡ Noyes, A. A., Jour. Amer. Chem. Soc. 27, 2, p. 85, 1905.

Colloidal solutions may exist in which the disperse phase may be found in other dispersion means than water. These with water are generally known as *sols*. When the dispersion means is water, the solution or suspension is specifically called hydrosol; in alcohol, alcosol; in glycerol, glycersol; etc. If the disperse phase takes up a certain amount of water, it may enter into a jelly-like condition when it is generally called a *gel*. In this instance, it would be called specifically a hydrogel. It is possible to have as well alcogels, sulphogels, etc. Gelatin may exist in a colloidal solution as a hydrosol and also as a hydrogel depending upon the amount of water employed. There also always exists the possibility of the disperse phase taking up some of the dispersion means and the dispersion means actually incorporating some of the disperse phase. To what extent this may be carried is problematical.

It has already been indicated that colloidal solutions differ from crystalloidal. The crystalloidal solutions are true molecular or ionic solutions. The molecule may or may not divide into ions. Sodium chloride passing into solution breaks into ions carrying with them a positive and negative electric charge which in turn create a current of electricity. The cane sugar molecule on the other hand does not break up but goes into a molecular solution; there are no positive and negative ions, consequently no electric dissociation. Substances which ionize as sodium chloride are called electrolytes while substances as cane sugar are non-electrolytes because they do not ionize. The colloids, too, like sugar, are non-electrolytes and do not ionize, yet they respond to a current of electricity passed through a solution. The particles of a colloid have a tendency to pass to one pole or the other depending upon the nature of the colloid. This reaction is called *electrophoresis*. Further, it may be said that, if colloids pass toward the anode, they are negatively charged, if toward the cathode positively charged. The significance of this movement of the particles of different colloids in response to an electric current passed through a solution does not seem to be clearly understood.

The size of the particles existing in a suspensoid or an emulsoid or even in a molecular solution is of considerable importance from the standpoint of stability, reaction to light and many other phenomena. Ostwald* presents the matter very tersely in the following diagram which has been slightly modified by the writers.

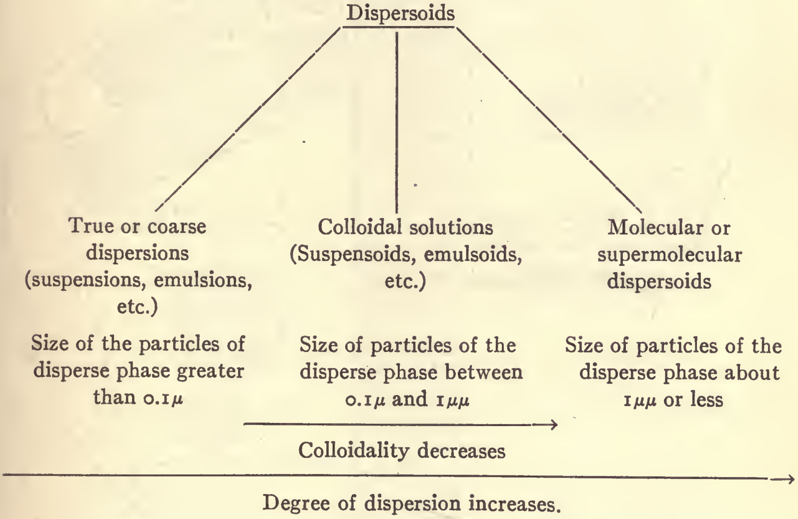
Dispersoids
True or coarse dispersions (suspensions, emulsions, etc.)
Size of the particles of disperse phase greater than 0.1μ
Colloidal solutions (Suspensoids, emulsoids, etc.)
Size of particles of the disperse phase between 0.1μ and $1\mu\mu$
Molecular or supermolecular dispersoids
Size of particles of the disperse phase about $1\mu\mu$ or less
Colloidality decreases
Degree of dispersion increases.

FIG. 107.—An arrangement of dispersoids. (*After Ostwald.*)

This graphic presentation can be still better understood by giving also the illustration provided on page 30 of the same publication (Fig. 8) of this publication (Fig. 108).

By use of the ultramicroscope developed by Siedentopf and Zsigmondy it has been possible to employ Tyndall's phenomenon which makes the visibility of rays of light passing through a medium dependent upon solid particles as dust in the air of a room. The light must enter into a dark room as a ray from one side only to illuminate the particles and render the demonstration successful. In the same manner particles suspended in a transparent medium may also be illuminated. The ultramicroscope makes it feasible to use Tyndall's phenomenon effectively in revealing particles of some colloidal substances and solutions having particles of larger dimensions. Siedentopf and Zsigmondy find that the microscope has its limitation of visibility at about 0.1μ and the ultramicroscope at about 1.0$\mu\mu$ (*submicron*) or 0.001μ. There are particles existing beyond the reach of the ultra-

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* Ostwald, Wolfgang. Handbook of Colloid Chemistry, p. 33.

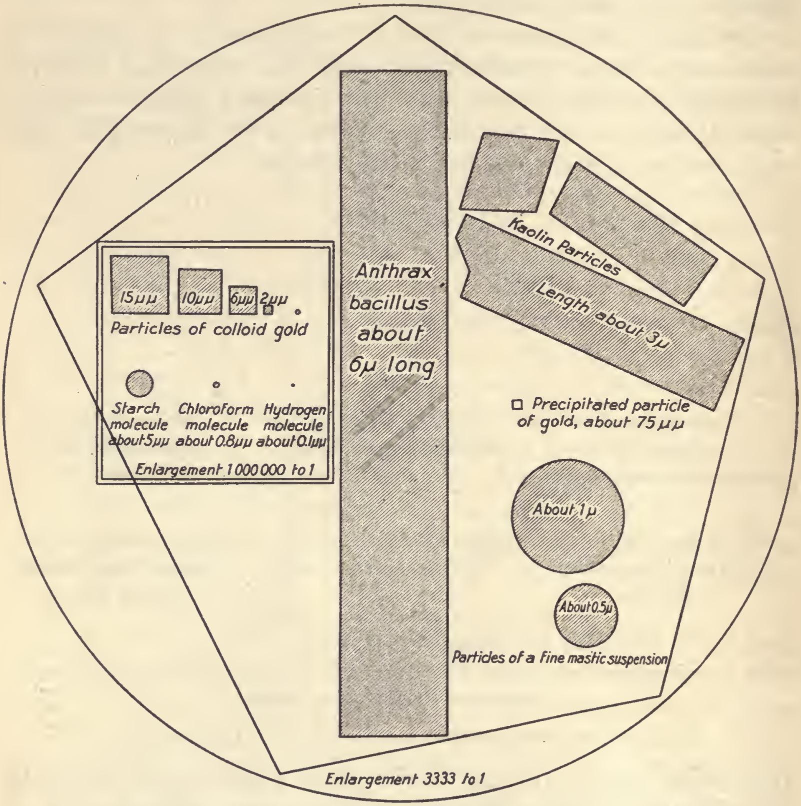
15μμ
10μμ
6μμ
2μμ
Particles of colloid gold
Starch molecule about 5μμ
Chloroform molecule about 0.8μμ
Hydrogen molecule about 0.1μμ
Enlargement 1000000 to 1
Anthrax bacillus about 6μ long
Kaolin Particles
Length about 3μ
Precipitated particle of gold, about 75μμ
About 1μ
About 0.5μ
Particles of a fine mastic suspension
Enlargement 3333 to 1

FIG. 108.—Comparison of particles of different sizes. (*Ostwald.*)

The large circle corresponds to the diameter of a human red blood corpuscle (about 7.5 μ); the large pentagon to that of a starch granule of medium size (about 7.0 μ). The particles enclosed in a frame are, in comparison with the rest of the figure, enlarged 333 times.

The figure has been constructed from data and tables given in *R. Zsigmondy* (Zur Erkenntnis der Kolloide, Jena, 1905). The values for the mastic suspension are taken from *J. Perrin's* studies [Kolloidchem. Beihefte 1, 221 (1910)].

microscope which are designated in size by the term *amicrons.* According to Zsigmondy the size of the particles covered in colloids ranges

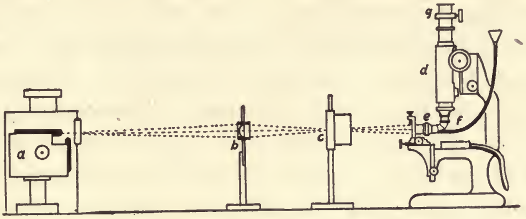

FIG. 109*a*.—Arrangement of ultramicroscope. (*After Bayliss.*)

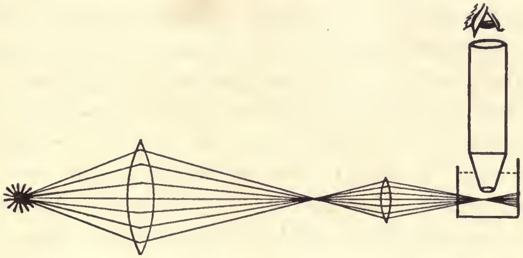

FIG. 109*b*.—Rays of light in ultramicroscope. (*After Bayliss.*)

from 0.1μ to 1μμ. Ostwald gives the estimated sizes of certain molecules:

Hydrogen gas	0.067–0.157μμ
Water vapor	0.113μμ
Carbon dioxide	0.285μμ
Sodium chloride	0.26μμ
Sugar	0.7μμ

Some conception of the size of molecules and colloidal particles, although they may not be absolute and even subject to great range or variability, contributes to an understanding of colloidal and molecular solutions, osmotic action, life-activities, lower limits of size of microorganisms and other natural phenomena.

The "disperse phase" of colloidal solutions suggests at once the extensive surface made possible by the particles in suspension and must likewise suggest the extent of surface energy present in the form of surface tension and adsorption. These factors are largely involved in colloidal reactions and life-functions. Their bearing has been already indicated (See 168).

It has already been said that Thomas Graham made the distinction between colloids and crystalloids by means of dialysis through a membrane, the colloids are withheld and crystalloids pass through. This movement on the part of these substances follows the laws of diffusion which, in turn, conform with the laws of expansion of gases. In the case where the membrane obstructs the movement of colloids and permits the crystalloids to pass there can be recognized an interference with free movement. Whether the colloidal molecule is larger than the crystalloidal molecule, which appears to be a fairly satisfactory undemonstrated reason, or not, does not materially alter the situation; or whether some chemical transition or obstruction accounts for this phenomenon of passage and check, in our present position, does not contribute much without a real working knowledge of what is involved. The facts remain: Colloidal substances do not pass while crystalloids do. This significant condition may be actually responsible for the cell-entities which incorporate the mechanism of life.

In colloids, diffusion is slow, slower than in the case of the crystalloids. This enables the crystalloids to penetrate or diffuse through the colloidal substances as protoplasm and sustain what must be regarded as a more or less fixed substance, protoplasm, through the very nature of its powers.

The microbial cell is generally a unicellular organism which secures its nutrition and performs its respiratory functions through the surface layer of the cell. This outer layer in most microbial cells takes the form of a membrane and where no membrane exists the cell seems to respond in much the same manner through its protecting surface layers of protoplasm. A yeast cell prepares its food which is not assimilable through its cell-wall by secreting suitable enzymes to produce diffusible nutrition. Such portions of this solution are assimilated through the cell-wall as are needed in cell-construction and are converted by similar processes within the cell substance while in transitional route to protoplasm itself. In the case of an amœba the particle of food is often taken within the protoplasm by means of its pseudopodia and after digestion is assimilated as in the yeast cell. This process in the amœba cannot be regarded as at all different from that of the yeast for the digestive-preparatory process and assimilation are much the same.

When food is prepared it is probably in the form of a molecular or ionic dispersoid which enters the substance of the protoplasm and diffuses readily. The ionization of the cell is, according to many authorities, dependent upon the ionic or molecular dispersoids which are found in the cell substance, whether they are on their way to become protoplasm or are the products of cell activity. When these ionic or molecular dispersoids of the cell are of a nature and possess the affinity to attach themselves to molecules of protoplasmic structure, their diffusibility is lost and they become anchored; if, however, there exist diffusible substances which are cast off from the protoplasmic molecules by metabolic action and no longer possess the affinity for attaching themselves, their dissipation by elimination is assured. The change of starch, glycogen, protein, as food, to diffusible products by regulation digestive processes and the elimination, as waste products, of diffusible substances have a tendency to confirm this vital interpretation.

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CHAPTER III

CHEMICAL STUDIES OF THE CONTENT OF MICROBIAL CELLS*

Microörganisms have a widely variable chemical composition. They differ so much in their requirements—their habits, their food needs, their moisture demands, their environmental atmosphere, and their capacity for change—that their great deviation from a constant nature, as manifested by superficial expressions, perhaps, does not awaken unexpected mental responses. They also undergo much alteration in their compositional nature as well as in their structural nature while passing stages in their individual developments. The vegetative or growing forms do not seem to have the same composition as the spore-forms or resting forms although it may be quite possible that fundamentally the exact composition exists in both and only more superficial substances are detectable; old cells differ from young cells and capsulated forms from uncapsulated forms. Food influences greatly the products found in protoplasm both quantitatively and qualitatively. While such products which are referable to food may not be strictly a part of what is contemplated in the composition of the cell, yet it is difficult many times to make the distinction. Doubtless most influencing agents whether external or internal have some power over the substances now recognized in cellular composition.

If, however, constancy in species is to be maintained, it is necessary to assume that there is to be found in every species a constant group or nucleus of chemical atoms or molecules whether existing independently or acting in consort in forming congeries of molecular complexes, and that substances fluctuating in their presence or in their amount must be regarded as more incidental to the basic life-processes. Species, therefore, even when undergoing all the recognized variations to which it is subjected—ageing, developmental stages, reproduction, environmental factors as food, reaction, oxygen supply, temperature, and others

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* Prepared by Charles E. Marshall and Arao Itano.

—remains basically constant, apart from its evolutionary possibilities, to its line of descent.

The student, too, should not be led to interpret the products found by the chemists as the substances constituting protoplasm or any of its differentiated parts but rather as substances entering into the formation of the protoplasmic molecule, or as substances resulting from metabolic processes, or as substances connected in some way with the food supply as reserve material or as substances essentially foreign, having entered the cell by means of its mechanical functional acts. Ultimate analyses may reveal the percentages of N, C, H, O, P, S and other elements; certain chemical methods may demonstrate the presence of proteins, amino acids, carbohydrates, and fats, and the ash may contain definite mineral constituents, yet such revelations are only the initial steps which will take the wandering industrious scientist or student to the museum of nature wherein are found the depicted substances and acts involved in living protoplasm. However, besides striving to obtain an insight into the very nature of life and its operating processes, much has been accomplished by such studies in ameliorating the conditions of man's existence and in helpfulness. By having even this very limited knowledge as will be gathered from the study of metabolism, soil, food, immunity and infectious diseases, extending to agriculture, medicine and the industries, great progress is possible and has been made.

ANALYSES

Moisture.—The moisture content of microörganisms has a very wide range. In the mother-of-vinegar made up largely of acetic bacteria, the moisture content reaches 98.3 per cent.; in *Bact. pneumoniæ*,* 85.55 per cent.; in the alga, *Chlorella vulgaris*,* 63.06 per cent.; in the spores of molds, 39 to 44 per cent. From this very brief survey it will be seen that all microörganisms vary greatly in their moisture content. The amount seems to be largely dependent upon the medium in which development takes place, unless it is in the case of spores which

* Nicolle, M., and Alilaire, E., in Ann. Inst. Pasteur, T. 23, p. 555, furnishes the following moisture determinations in per cent.: *Bact. mallei*, 76.49; *Bact. choleræ gallinarum*, 79.35; *Msp. comma* (Bombay), 73.38; *Bact. dysenteriæ* (Shiga), 78.23; *Proteus vulgaris* (*B. proteus*), 79.99; *B. typhosus*, 78.93; *Bact. anthracis* (asporogenic), 81.74; *Bact. pseudotuberculosis*, 78.83; *Bact. pneumoniæ*, 85.55; *B. coli*, 73.35; *B. prodigiosus*, pathogenic (de Fortineau), 78.00; *B. psittacosis*, 78.05; *Bact. diphtheriæ*, 84.50; *B. pyocyaneus*, 74.99; *B. lymphangitis* (de Nocard), 77.90; yeast (Frohberg), 69.25; *Chlorella vulgaris* (alga), 63.6.

incorporate an amount which is difficult to remove and which has some relation apparently to their high degree of resistance.

Molds have, as a rule, a greater moisture content than yeast and yeast a greater content than bacteria, yet these organisms have no constancy or uniformity in their moisture content. The protozoal forms are as dissimilar as others and their range of moisture content assumes no fixed boundaries.

Although there is a minimum limit and a maximum limit as indicated on the one hand by desiccation and on the other hand by an inability to absorb more moisture, still retaining life one is forced to believe in a very restricted amount of moisture as essential to life-processes. Beyond this essential amount, in the case of too little, the metabolic activities cannot take place, and, in the case of an excessive amount, proper functioning is interfered with or a modification of physiological reactions gradually becomes more and more evident.

Proteins and other nitrogenous substances.—Nitrogenous compounds are present in varying amounts and are assumed to be the basis of protoplasm. The approach in the study of this class of substances has been made through the determination of nitrogen, then converting the nitrogen into terms of protein by the use of the recognized factor; by the recognition of definite nitrogenous compounds which may represent certain portions of the protein molecule; and by the use of reagents long employed to detect the presence of protein, largely qualitatively. All of these can furnish only inadequate means for the recognition of the nitrogenous materials which may enter into the formation of the active life-substance, protoplasm. However limited may be the knowledge available in this particular subject, there is now at hand sufficient to point the way for more and for certain directive practical purposes. The per cent. of nitrogen[*] found by Vaughan and his associates and by Nicolle and Alilaire ranges from 3.96 (dry weight,

[*] Vaughan and Wheeler. "Protein Split Products in Relation to Immunity and Disease," by Vaughan, contributes the nitrogen determinations in per cent. for several bacteria: Typhoid, 11.55; colon, 10.65; tuberculosis, 10.55; anthrax, 10.285; subtilis, 5.964; *Proteus vulgaris*, 6.791; Ruber of Kiel, 10.655; megaterium, 8.349; pyocyanus, 10.843; violaceus, 11.765; *Sarcina aurantiaca*, 11.46.

Nicolle, M., and Alilaire, E., in Ann. Inst. Pasteur, 23, 555, give the following nitrogen results in per cent. (based upon dry weight), *Bact. mallei*, 10.47; *Bact. choleræ gallinarum*, 10.79; *Msp. comma* (Bombay), 9.79; *Bact. dysenteriæ* (Shiga), 8.89; *B. proteus* (*Proteus vulgaris*), 10.73; *B. typhosus*, 8.28; *Bact. anthracis* (asporogenic), 9.22; *Bact. pseudotuberculosis*, 10.36; *Bact. pneumoniæ*, 8.33; *B. coli*, 10.32; *B. prodigiosus* (pathogenic) (de Fortineau), 10.55; *B. psittacosis*, 9.55; *B. pyocyaneus*, 9.79; *B. lymphangitis* (de Nocard), 9.17; yeast (Frohberg), 10.00; *Chlorella vulgaris*, 3.96.

in *Chlorella vulgaris* (alga) to 10.73 in *B. proteus* (*Proteus vulgaris*). In the protozoön, *Noctiluca miharis*, there was present 7.74 per cent. of nitrogen as determined by Emmerling.[*] Molds and yeasts appear to lie between the alga named and many of the bacteria as indicated by the work of Marschall and Nageli.[†]

The compounds of nitrogen which have been determined are quite numerous although it must be allowed that the analyses have not always been satisfactory. Ruppel[‡] claims to have determined nucleic acid, nucleoprotamin, nucleoproteid, albuminoids (keratin, etc.) in dried *Bact. tuberculosis*. Nishimura[||] found nuclein bodies as xanthin, guanin, adenin in a water bacillus. Vaughan[§] and his associates have been able to demonstrate the presence of various amino acids. The work of Emmerling[*] also contributes much which aids in our understanding of definite substances in the protoplasm of protozoa.

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[*] Emmerling, O., Biochem. Zeitschr., 1909, gives this analysis of *Noctiluca miliaris*: In 100 grams of ash free substance there was 7.74 grams of nitrogen (Taken from S. von Prowazek: Physiologie der Einzelligen.)

Lysin	0.212	with 0.040 grams nitrogen
Arginin	1.6492	with 0.432 grams nitrogen
Histidin	3.4762	with 0.938 grams nitrogen
Tyrosin	0.5271	with 0.041 grams nitrogen
Glycocoll	15.9000	with 2.956 grams nitrogen
Alanin	2.4000	with 0.378 grams nitrogen
Leucin	0.4200	with 0.044 grams nitrogen
Prolin	4.6000	with 0.556 grams nitrogen
Asparagin acid	0.1700	with 0.020 grams nitrogen
Total		5.405 grams nitrogen.

[†] Marschall, Arch. f. Hyg., 28, 19, estimates the protein in *Aspergillus* at 30.4 per cent., in *Penicillium* at 40.2 per cent., and *Mucor* at 43.4 per cent. (based upon dry weight). In Arch. f. Hyg. 28, 1917, 17, the per cent. of protein in molds is placed at 38.0.

Nageli and Loew., Jour. Prakt. Chem. N. F., 17, determined 47.0 per cent. of protein in yeasts.

[‡] Ruppel. Zeit. f. Physiol. Chemie, XXVI, 1898, out of 100 grams of dried *Bact. tuberculosis* secured the following substances:

Nucleic acid (tuberculinic acid)	8.5 grams
Nucleoprotamin	25.5 grams.
Nucleoproteid	23.0 grams
Albuminoids (keratin, etc.)	8.3 grams
Fatty matter	26.5 grams
Ash	9.2 grams

[||] Nishimura, Arch. f. Hyg. XVIII, 318, 1893, reports the finding of 0.17 per cent. xanthin, 0.08 per cent. adenin and 0.14 per cent. of guanin in his water bacillus.

[§] Vaughan, V. C. and associates, loc. cit., have noted the presence of certain diamino and monamino acids.

The protein substances vary in amount in different species of microörganisms. Vaughan[*] compares the compounds of *B. coli* and *Bact. tuberculosis* indicating that no similarity of amino acids exists in the protoplasm. Duclaux[†] has found in the analysis of yeast, 15 years old, only 2.7 per cent. of nitrogen as compared with the yeast (Frohberg) analyzed by Nicolle and Alilaire which contained 10 per cent. nitrogen. Age, it seems from this, changed the amounts of nitrogenous material present in the cell. Then, again, the medium upon which the microörganisms are cultivated has a decided influence. Cramer[‡] determined 69.25 per cent. protein in *Msp. comma* when grown in bouillon and only 35.75 per cent. when grown in Uschinsky's solution. He also noted that the dry matter from this organism was greater when grown at body-temperature than when grown at room-temperature.

Carbohydrates.—Substances which correspond to the reactions of carbohydrates have been recognized. Some of these substances exist as distinctive carbohydrates and some enter into the formation of compounds as glyco-proteins. Their relation to the protoplasmic molecular structure and to nutritive processes is still more obscure.

Glycogen has been reported by A. Fischer[||] in *B. subtilis* and *B. coli.* Levene[§] has found it in *Bact. tuberculosis.* Marschall in the study of molds records the presence of 3.7 per cent. starch. However, glycogen is so much like starch that confusion has arisen. Glycogen in molds and yeasts, much like that of animal glycogen, is claimed by several workers. (Glycogen has been commonly known as animal starch from the time of Claude Bernard.) In protozoa glycogen has been determined by Sosnowski[¶] in *Paramecium* and by Bütschli in *Gregarina.*

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[*] Vaughan, V. C. and his associates, loc. cit., compare the amino acids of *B. coli* and *Bact. tuberculosis.*

	B. coli, Per cent.	*Bact. tuberculosis,* Per cent.
Glutamic acid	3.00	0.20
Glycocoll	0.33	0.00
Alanin	1.00	1.40
Valin	1.60	4.60
Leucin	2.00	1.82
Phenylalanin	0.20	0.50

[†] Duclaux, E.: Kruse, "Allgemeine Mikrobiologie," p. 59.

[‡] Cramer, E., Arch. f. Hyg. 28, 1.

[||] Fischer, A.: Vorlesungen über die Bakterien, Jena, 1903.

[§] Levene, Jour. Med. Research, 6, 135, 1901. Scheibler, Zeitsch. f. Rubenzuckerindustrie XXIV, 309, 1874. Marschall, Arch. f. Hyg., 28, 19, 1897.

[¶] Sosnowski, Centralblatt f. Physiologie, 13, 1899.

Cellulose, so bound up with plant life and at one time so much used to differentiate plant and animal life, has not been positively demonstrated in any microörganism, even in molds and yeasts. Substances, giving suggestive reactions, have been studied and, at times, have been called cellulose, or some modified form of cellulose, yet recent analysts seem to think there is really no substantial ground for this assumption. Vaughan* in his extensive analyses of bacterial cells has never been able to identify cellulose. On the other hand Vaughan calls attention to two carbohydrate bodies, one of which furnishes a reducing sugar when boiled with dilute mineral acid and the other does not.

From time to time there have been detected suggestive traces of various carbohydrate substances to which special names have been attached but they seem to lack definiteness and individuality in their chemical features. Chitin,† a substance quite generally found in microbial cell-walls, consists apparently of a carbohydrate-amine or glucosamine polymerized. Much emphasis is now placed upon this substance as representing the most important constituent not only of microbial cell-walls but of wings and coverings of insects and of many lower animal forms.

Fats.—Many analyses indicate variable amounts of fat in all classes of microörganisms. Whether this fat is the result of degradation processes at times, whether it may be ready for assimilation, whether it exists as a reserve product, or whether it is the yield of direct absorption cannot be asserted off-hand. Probably there are times when it may answer to each of these explanations and times when indications are such as to furnish a positive understanding.

Fat globules may be readily revealed by the use of certain stains as osmic acid and Sudan III when present in comparatively large microbial cells, but in the case of bacterial cells this procedure is unavailing, making it necessary to employ recognized chemical methods.

In the analysis of molds, Marschall‡ has obtained the following

	Aspergillus	*Penicillium*	*Mucor*
Ether extract	4.7	4.1	4.0
Alcoholic extract	18.5	11.8	11.8

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*Vaughan, V. C. and his associates, loc. cit.

†Chitin when hydrolyzed yields glucosamine and acetic acid. The equation $C_{18}H_{30}N_2O_{12} + 4H_2O = 2CH_2OH.CHOH.CHOH.CHOH.CHNH_2.CHO + 3CH_3COOH$, has been suggested.

‡Marschall, Arch. f. Hyg., 28, 19, 1897.

sodium occupy very prominent places; yet the relations of these two elements are sometimes reversed. Calcium and the other constituents are subject to considerable fluctuation. If any inference is to be drawn from this work, it must mean that phosphorus is a very important element, serves an essential rôle, and is of consequence to protoplasm, probably as a basic constituent. Potassium, sodium, magnesium, and calcium are uniformly constant ingredients, are concerned in nutritional exchanges and may in a limited manner be bound in the structure of the protoplasmic molecule.

The concentration of the culture-medium and brine solutions are known to influence the amount of ash-content of microörganisms. Cramer,* using a 1 per cent. sodium carbonate bouillon, a 4 per cent. sodium phosphate bouillon and a 3 per cent. sodium chloride bouillon obtained in the case of *Msp. choleræ* respectively 9.3 per cent., 22.3 per cent., and 25. 9 per cent. ash (dry weight).

Other substances are found present in microbial cells. These should be referred to here although more extensive consideration will be given some of them later.

Enzymes are found in all microbial cells. They are agents employed in metabolism and in the preparation of food for incorporation in the body of the cell and incidentally produce changes which result in products of fermentation as alcohol. They act very specifically inasmuch as a particular enzyme is needed for every substance changed as cane sugar, malt sugar, starch, protein, fat, etc. They cause change apparently without altering their nature. They are influenced by many conditions of temperature, reaction, accumulated products, etc. An organism is capable of secreting or containing within its protoplasm several enzymes, each being produced only when the cell is specifically stimulated.

Toxins much like enzymes may be found within the cell substance or in the medium in which the microörganism may be growing. They are associated with disease-production and pathogenesis. Their force as a poison (the meaning of the word) is incomparably great. Only a small number of microörganisms are able to produce toxins.

Vitamines are substances, somewhat intangible, which have been found in some microörganisms and quite generally in food substances. They are seemingly essential to life. Their recognition at the present time is largely by solubility and physiological determination upon animals.

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* Cramer, Arch. f. Hyg , 28, 1.

DIVISION II

NUTRITION AND METABOLISM

INTRODUCTION*

The nutrition and metabolism of microörganisms are based on many of the same principles which regulate animal and plant metabolism; in many ways microörganisms are more closely related to animals than to plants, if viewed from the standpoint of their food, their mode of digestion, and their general physiological nature. Aside from the many specific physiological processes peculiar to microbial life as in the case of life without oxygen (anærobiosis) and in the ability of some species to use free nitrogen gas, the functioning of microörganisms accords with the cellular metabolism and nutritive principles of the more highly developed organisms. Since it will be desirable frequently to refer to plant and animal nutrition in the course of this discussion, these principles, therefore, are briefly discussed in the following paragraphs.

Green plants feed only on inorganic substances. They assimilate carbon dioxide (CO_2) from the air which unites with water, nitrates, potassium, calcium, and other salts of the soil and form the body substances of the plant. The cellulose, starch, sugar, protein and all other compounds constituting the plant cells are produced from these simple inorganic substances. Animals feed upon animals and plants. Unlike plants they utilize the oxygen of the air and give off carbon dioxide (CO_2). Out of these materials, together with water, life is sustained. Although in details animals, plants and microörganisms differ quite widely, the general laws of nutrition and metabolism are very similar.

The methods by which microörganisms secure their food vary. Molds take up their food through the mycelium after it has been prepared by the action of digestive agents, enzymes, secreted by the cells. If the food be suitable for the life of the cell without change, of course, these digestive agents are not needed. When properly altered, such sodium occupy very prominent places; yet the relations of these two elements are sometimes reversed. Calcium and the other constituents are subject to considerable fluctuation. If any inference is to be drawn from this work, it must mean that phosphorus is a very important element, serves an essential rôle, and is of consequence to protoplasm, probably as a basic constituent. Potassium, sodium, magnesium, and calcium are uniformly constant ingredients, are concerned in nutritional exchanges and may in a limited manner be bound in the structure of the protoplasmic molecule.

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* Prepared by Otto Rahn. Revised by Editor.

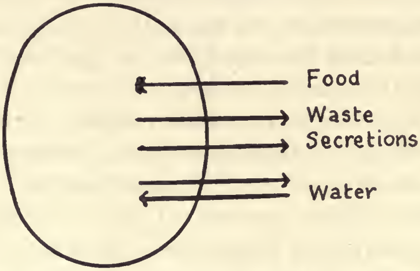

FIG. 110.—Illustrating cell activities.

Waste products resulting from the metabolism of protoplasm leave the cell through the cell-wall, also by means of osmosis, and this process appears to be the same for the ingestion of food as for the egestion of waste products.

Some microörganisms live upon dead matter, some upon living matter and some may make use of either. The greater portion, by far, require or prefer organic substances. When organisms, as protozoa, feed upon living organisms they are said to be *holozoic* in their mode of life, in other words they follow closely the methods employed by animals. Then there are those protozoal organisms which simulate plants in their manner of nourishment. These are called *holophytic*. This latter class is associated with the formation of chlorophyll-bodies within their structure. There are those organisms, too, which consume organic matter which is rendered suitable by nature or decay, called *saprozoic* or *saprophytic*, depending upon whether the organism is designated as animal or plant. Whenever organisms require living tissues to sustain life, in the form of a host, they are called *parasitic*.

Many of these microörganisms absorb their nutrition directly from the fluids of the tissues while others, amœbæ, are able to devour cells.

Protozoa are very much like all microörganisms in their manner of living but there are details which belong to them as a class and should be pointed out specifically.

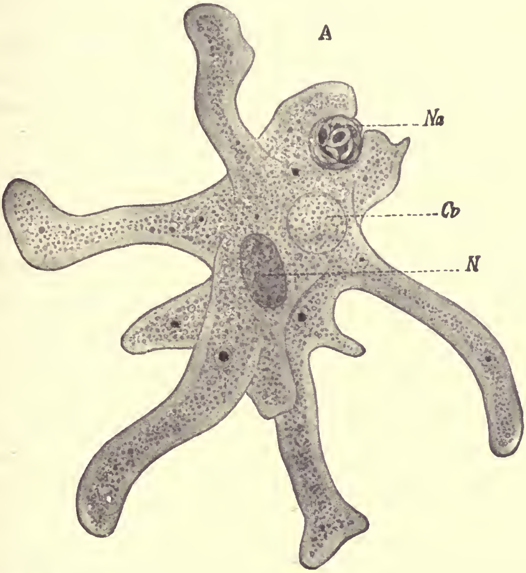

FIG. 111.—*A, Amœba proteus; Na,* a food particle; *Cv,* contractile vacuole; *N,* nucleus. (*After Doflein.*)

*"The ingestion of food is accomplished in some protozoa by pseudopodia; the protozoön simply flows around and so encloses a food particle (Fig. 111). In the same way, these protozoa flow away from waste particles which are to be eliminated. Other protozoa have definite mouth areas for the ingestion of food, and definite anal areas for the discharge of residual material. Those protozoa which ingest solid food, digest it within gastric vacuoles by the aid of enzymes and of acids, just as is the case in many-celled animals. The most important of the disease-producing protozoa live within nutrient fluids, for example the blood, and they obtain their nourishment from the fluid in

which they live, by osmosis; consequently, they have no definite mouth area, nor gastric vacuoles.

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* Prepared by J. L. Todd.

*"Some of the protozoa, for example, some amœbæ and ciliata, possess contractile vacuoles. A contractile vacuole is a clear cavity which appears in the cytoplasm, grows slowly, empties itself by a rapid contraction of the fluid which has drained into it and forms again. The fluid which it ejects contains the soluble waste products resulting from the metabolism of the protozoön. One function of the contractile vacuoles is, therefore, excretion; in some protozoa, they are probably also concerned with respiration. Contractile vacuoles are usually absent in protozoa which are parasitic within other animals.

*"The process of respiration in the protozoa is in general similar to that of higher animals. Most of them require oxygen and eliminate carbon dioxide. The contractile vacuole which is found in certain forms is believed to have a respiratory function. Respiration may consist of the liberation of energy through oxidation or through the breaking down of complex molecules. In organisms of an anaerobic habit the respiration is probably through internal molecular changes affecting material stored in the cytoplasm.

*"In addition to the expulsion of solid undigested material from the cytoplasm there is evidence that waste products other than CO_2 are excreted by contractile vacuoles. Many organisms also secrete material either of the nature of chitinous membranes on their surface or metabolic products in the form of granules, etc., within their bodies.

*"Derangement of function may be produced associated with it are visible degenerative changes. It has also been found that certain protozoa have the ability to recover from injury and to regenerate lost parts."

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* Prepared by J. L. Todd.

CHAPTER I

ENERGY REQUIREMENTS IN CELLULAR NUTRITION*

The formation of organic compounds from inorganic compounds requires a certain amount of energy. If a certain quantity of sugar is burned to carbon dioxide (CO_2) and to water (H_2O), a certain amount of energy is liberated in the form of heat. The heat given off in this case is also a distinct product of combustion. This heat is always obtained in the same amount regardless of the method chosen in burning the sugar. It has been definitely determined to be 674 calories for 1 g. molecule (180 g.) of sugar. The complete equation of sugar combustion is therefore written

$$C_6H_{12}O_6 + 12O = 6CO_2 + 6H_2O + 674 \text{ Cal.}$$

Consequently the same amount of energy will be needed to produce sugar from carbon dioxide and water; for the law of the conservation of energy requires that, if a certain process liberates a certain quantity of energy, the reverse process will require the same quantity of energy. Green plants get their energy from the sunlight; exactly the opposite proceeds in the equation which should read from right to left; CO_2 and H_2O are absorbed by the plant resulting in the formation of sugar. But it is evident from the equation that CO_2 and H_2O are not sufficient to produce sugar since it takes 674 calories of heat in addition. The radiant energy of light is transformed by the chlorophyl granules of the plant leaves into chemical energy which causes the formation of organic compounds from the simple inorganic or mineral matter. Chlorophyl is the green coloring substance of plants, and only green plants can use the energy of sunlight for their growth.

The growth of green plants is a storing of the energy of light in the form of organic matter; their metabolism is largely synthetic, *i.e.*, building up. Plants without chlorophyl, however, like mushrooms, molds, yeasts and bacteria, have to provide for their energy by some other means.

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* Prepared by Otto Rahn.

Animals construct their bodies mainly of organic matter. Their body substances as protein, fat, etc., are derived from the protein, fat, cellulose, etc., of plants or of animals. Nevertheless, a certain amount of energy is required in this assimilation process, since the animal protein and fat are somewhat different from the plant protein and fat. Consequently, complex chemical changes and rearrangements, which require some energy, are necessary for growth. Energy is also lost by radiation of heat and by locomotion. Animals, being entirely unable to use the sunlight as a source of energy, obtain their energy from the digestion of organic food. The larger part of this food is oxidized completely; this part provides the energy. The smaller part of the food is used for building the tissues of the body; it becomes part of the animal itself. Animal metabolism is largely analytic, *i.e.*, destructive although a limited amount of energy is required for the chemical changes and molecular rearrangements which are essential to animal tissue formation—a synthetic process. Accordingly more organic matter is decomposed than is formed. Often the same substance can serve both purposes; the meat eaten by a dog furnishes to it energy as well as material for growth. In other cases, certain food compounds execute only one function and not the other. This distinction between food for energy and food for growth must also enter into the interpretation of microbial metabolism.

It might appear from this discussion that energy is needed only by growing cells, as the full-grown cells do not increase in size or weight or number. They also need energy, for in all living cells, there is noticed a continuous breaking down (*katabolism*) and rebuilding (*anabolism*) of the cell constituents. This process is commonly called *metabolism*. The katabolic processes (the breaking down) in a cell will continue even if the cell receives no food. The cell loses in weight and the starvation which follows will ultimately result in the death of the cell. All living cells require food for the maintenance of life.

In the first part of this book, microörganisms have been divided into plants and animals, but attention has been called in various places to the fact that it is often hard to determine whether the plant characters or the animal characters prevail. This holds true not only with the morphology, but also with the physiology of microörganisms. Since none of the plants discussed in this text-book possesses chlorophyl, none of them can use light as a source of energy, therefore they depend entirely upon chemical energy obtained by the digestion of food. This means that they require organic food almost entirely, since inorganic food furnishes energy only in exceptional cases. In this respect they resemble the animals very much.

The source of energy in microbial life is always of chemical origin. The simplest processes are the oxidations, and simplest among these the inorganic oxidations. A number of different types feeding exclusively on minerals has been discovered during the last twenty years, and some of them are of great economic importance. They resemble plants in as far as they build their cells exclusively from carbon dioxide, nitrates and ash. The food used for building material is quite different from the food used for the provision of energy.

Two typical examples are the nitrifying organisms in soil which oxidize ammonia to nitrates. This process, according to Winogradski, is divided distinctly into two phases: the *Nitrosomonas* oxidizes the ammonia to nitrous acid,

$$NH_3 + 3O = HNO_2 + H_2O + 78.8 \text{ Cal.}$$

and the *Nitromonas* oxidizes the nitrous acid to nitric acid,

$$HNO_2 + O = HNO_3 + 18.3 \text{ Cal.}$$

These oxidation processes yield a certain amount of energy which enables the bacteria to build their cells from carbon dioxide, ammonia, and certain mineral salts. Without ammonia or without nitrous acid, respectively, these bacteria cannot grow for lack of energy; they would be like a plant without light. It is evident in this case that the food for energy is also used to some extent as food for growth. The nitrogen necessary to the bacteria is supplied by the ammonia or the nitrous acid.

As an example distinguishing strictly between the food for growth and the food for energy may be mentioned the hyposulphite bacterium studied by Nathanson. This organism oxidizes hyposulphites to sulphates and sulphur, largely following the formula

$$\underset{\text{Hyposulphite}}{Na_2S_2O_3} + O = \underset{\text{Sulphate}}{Na_2SO_4} + \underset{\text{Sulphur}}{S} + x \text{ Cal.}$$

Besides, some more complex compounds, like sodium tetrathionate ($Na_2S_4O_6$), are formed. The bacterium builds its cells exclusively from nitrates, carbon dioxide, and mineral salts; organic food is rejected. The hyposulphite can hardly be used for the construction of the cell, and must be considered entirely a food for energy.

This distinction is not confined to mineral decomposition only. The urea bacteria get their energy from the decomposition of urea into ammonium carbonate which is hydrolysis.

$$\underset{\text{Urea}}{(NH_2)_2CO} + 2H_2O = \underset{\substack{\text{Ammonium}\\\text{carbonate}}}{(NH_4)_2CO_3} + 14.3 \text{ Cal.}$$

But the urea and mineral salts are not sufficient for the development of the urea bacteria. They cannot use urea as a material for building the cells, and they cannot use carbon dioxide or carbonates; they cannot grow unless a suitable material for cell construction is added. Söhngen demonstrated that a few milligrams of malic acid favor a good development of the bacteria. The malic acid is used entirely for the formation of cell substances. The energy for this formation came from the urea fermentation. This example shows clearly the different requirements for cell growth and for the energy supply.

With the urea fermentation, we have changed not only from inorganic to organic food, but also from oxidation processes to other decompositions.

Microörganisms differ from the higher animals by their less complete metabolism. The food in the animal, if digested at all, is oxidized as a rule to the final products of combustion, CO_2 and H_2O, the only exception being the nitrogen which leaves the body still in organic combination as urea. With bacteria, yeasts and molds, this is not always the case. Though some of these organisms will bring about complete oxidation of the food we find more commonly incomplete oxidations or changes which require no oxygen at all, but still yield energy to the cell. The biochemical side of these changes of which the alcoholic fermentation is the best known will be discussed in the chapter on oxygen requirements.

CHAPTER II

MECHANISM OF METABOLISM*

General Theory of Metabolism

Anabolism, Katabolism, Metabolism.—It has been stated that microörganisms need food for at least two different purposes: building material and building energy. They may need it for other purposes also, *e.g.*, for motion. The sum of all changes which the food undergoes in the body, including the deterioration of the cells, is called *metabolism.* Metabolism consists of several separate functions: One of them is the construction of new cells, or parts of cells, called *anabolism,* another the deterioration of cells, called *katabolism,* and the most important quantitatively is the *fermentation* or *respiration.* The fermentation or respiration processes are fairly well understood; many of them can be produced in the chemical laboratory without microorganisms. Katabolism is the sum of many processes some of which are well understood while others are still unknown. The synthetic, anabolic processes of the cell, however, are almost entirely unknown, and we can only speculate regarding the various means by which the cell grows. The explanations of the different cell activities began, as in most other fields of theoretical microbiology, with a close analogy with animal and plant metabolism, but owing to the comparative simplicity of the microörganisms, they led to the establishment of new facts and theories which proved afterward useful for the understanding of the metabolism of the more complex organisms where the multiplicity of facts prevented a clearer insight into the separate processes.

Intra- and Extra-cellular Fermentation

Decomposition of Insoluble Food.—Many microörganisms feed upon cellulose, starch, fat, gelatin, keratin and other insoluble compounds. Microörganisms, with the exception of some protozoa, depend upon soluble food since they have no means of incorporating insoluble compounds into their protoplasm. The protoplasm, however, must be considered the center of metabolism, and the digestion of food and the formation of energy must take place in the protoplasm if the cell is to profit by it. Since the food cannot diffuse into the cell, and the protoplasm does not diffuse out, the food must be dissolved. This is accomplished by the cell itself by secreting certain agents with peculiar qualities. These agents, the so-called *enzymes*, act upon the insoluble foods, changing them into soluble compounds which then can diffuse into the cell where they are digested or fermented. The final digestion or fermentation of the food must take place within the cell. Energy production outside the cell serves the same purpose as a stove outside the house. The dissolution of insoluble compounds by cell secretions must be considered a preparatory process which has no direct relation to intra-cellular food digestion or fermentation. Enzymes are not produced by microbial cells exclusively. All living cells produce enzymes. They were known before the science of microbiology had been established. In fact, microbial activity was considered for a long time as an enzymic chemical process. Enzymes in the animal and plant body serve largely the purpose of metabolic changes. In the animal body, many enzymes help to dissolve the insoluble food which cannot pass from the alimentary canal into the body except by diffusion through the mucous membrane. There is *diastase* in the saliva which acts upon starch, there is *pepsin* in the stomach and *trypsin* in the intestine, both dissolving protein bodies; there is *ereptase* for the peptones, *lipase* for the fat, *invertase* for the saccharose, and many other enzymes. The object of all these enzymes is apparently to prepare the food for passing through the membrane into the protoplasm of the cells, where the final changes which liberate energy take place. The same processes occur with microörganisms but in a more simple manner. Surrounded by a liquid medium, they secrete enzymes; these dissolve certain insoluble foods which then diffuse through the cell wall to be decomposed further.

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* Prepared by Otto Rahn.

The food-preparing processes are all supposed to be simple hydrolytic processes. For some of these changes the chemical equations are well known. The hydrolyzation of starch to maltose by means of diastase is represented by the equation

$$2(C_6H_{10}O_5)_n + nH_2O = nC_{12}H_{22}O_{11}.$$

The splitting up of a fat molecule into glycerin and fatty acid is also a well-known process

$$\underset{\text{Tristearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = \underset{\text{Glycerin}}{C_3H_5(OH)_3} + \underset{\text{Stearic acid}}{3C_{18}H_{36}O_2}.$$

Proteolysis is not so well known and the general supposition that the first stages of protein degradation are hydrolytic is largely based upon analogies. Some of these enzymes which are secreted by the microbial cells act upon soluble compounds. *Invertase* decomposes saccharose into dextrose and levulose:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

Other disaccharides are hydrolyzed in the same way by other enzymes; glucosides are decomposed by *emulsin;* soluble proteins are changed to peptones. It is not necessary that the enzymes act upon the soluble compounds outside the cell since these compounds can diffuse into the cell; these enzymes are found only occasionally within the cell. It may be said, however, that the smaller molecules of the products of enzymic action diffuse more readily than the larger molecules of the original food compound.

PROPERTIES OF ENZYMES.—These secretions of cells are treated in a group by themselves because they differ distinctly in many respects from any other chemical substance. Probably the most notable difference may be discovered in the fact that their action does not follow the law of mass action which supposes that all substances reacting upon each other diminish in quantity. *Rennet* will coagulate many hundred times its weight of casein, and still the whey will contain rennet. Considering that part of the rennet is physically absorbed by the coagulum, the amount of rennet is found to be the same as before, though it has changed a comparatively enormous quantity of casein. The same is true with other enzymes. The enzyme is not destroyed by acting upon other substances. This exceptional quality furnishes a reason for treating enzymes as a separate group or apart from other chemical substances. But there are still other qualities which distinctly separate them from the well-known chemical bodies, and show at the same time their relation to proteins and toxins (page 248). One of these is their sensibility to such outside influences as will destroy life. Enzymes are inactivated by exposure to temperatures above 50° to 80°, and can, like coagulated albumin, by no means be brought back to their original state. This temperature is very near the coagulating temperature of albumin. It is believed from this resemblance that enzymes are of an albuminous nature. Another similarity is the fact that both enzymes and albumins are precipitated by concentrated salt solutions. Enzymes can further be inactivated by poisons. The same substances which kill living cells, like formaldehyde, hydrocyanic acid, mercuric chloride, phenol, will also inactivate enzymes, though usually stronger solutions are required for the destruction of the enzyme than for killing the cell. It is the same with heat; a higher temperature is generally required to destroy the enzyme than to kill the cell which secreted it. Light will also affect enzymes considerably. The great similarity of enzymes and microörganisms in these respects, the similarity of their reactions and the extreme minuteness of the bacteria render it explicable why the chemists of eighty years ago could not determine the difference between microörganisms and enzymes, and called them both "ferments."

With the toxins, the enzymes have in common the great sensibility to heat, light, and chemicals. Both of these groups are resistant to drying to a limited extent. So far as body reactions are concerned these two groups seem to belong to one physiological group of compounds. When toxins are injected, the body responds by the production of antitoxins which inactivate the toxin. In the same way the body responds to enzymes by the production of anti-enzymes which prevent the action of the enzymes. It may be mentioned that against protein compounds, precipitins are produced by the body which precipitate only that protein which was injected. This "specific" action is also true with toxins and enzymes. The anti-body will inactivate only the specific kind of toxin or enzyme that was injected.

What an enzyme really is cannot be defined. An enzyme is known only by its reactions. Many chemists have tried to prepare pure enzymes by continuously dissolving and precipitating, by dialyzing and other means, but there are two great difficulties existing; there is no test for the purity of enzymes, and they lose in activity if treated with chemicals. The more they are freed from the protein bodies which always accompany them, the more sensitive they are to injurious influences. Mineral salts seem essential for their action, because continued dialyzing weakens the activity which can be restored only by adding salts.

ENZYMES OF FERMENTATION.—It has been demonstrated in the above paragraph that food is prepared for digestion or fermentation by enzymes. The final decomposition, the process which yields the energy for cell life, must take place within the cell.

The difference in importance of food preparation and fermentation may be illustrated by the example of *Rhizopus oryzæ*. This mold attacks starch, changes it, by means of diastase, to maltose, the maltose to dextrose, dextrose to alcohol and carbon dioxide. The mold grows well in a starch medium, without sugar; it grows equally well in maltose, and equally well, or better, in dextrose; it does not grow at all with alcohol and carbon dioxide. The last change, dextrose to alcohol, is absolutely necessary for this organism; it is the source of its life; the others are incidental processes, not absolutely necessary under all circumstances, in fact greatly suppressed if dextrose is given together with starch. The fermentation must take place in the cell; the preparation of food may take place in the cell or outside; it is not essential where it happens.

The investigations of recent years have demonstrated that fermentations also are caused by enzymes. It has been proved beyond doubt that in the alcoholic, lactic, acetic and urea fermentations the fermentation process may continue after the death of the fermenting cells. In the case of alcoholic fermentation, the fermenting agent was separated first by Buchner from the lacerated cells and was filtered through porcelain filters without losing its ability to act. This proves the enzyme-nature of the fermenting agent which, once being formed, remains and acts independent of the cell. These enzymes are called *zymases*. They remain within the cell as long as it is alive. They are much more sensitive to injurious influences than the above-mentioned food-preparing enzymes. Much skill and patience was required to demonstrate their independence of the living cell. After these enzymes were found in microörganisms, similar enzymes were discovered in the cells of higher plants and animals. Many of the biochemical changes taking place in the final dissociation of food within the cell are known to be the result of enzymic action; heretofore these reactions were believed to be a part of the life processes, inseparable from the living cell. Even some of the oxidations and many reducing processes have been recognized as caused by enzymes, and it is quite probable that the whole process of intracellular food decomposition in all organisms is accomplished entirely by means of enzymes.

GROUPING OF ENZYMES

Since the chemical nature of enzymes and of their action is largely unknown, they can be arranged for convenience only according to the compounds they act upon. It is possible, however, to distinguish between the following four groups: *Hydrolyzing*, *zymatic*, *oxidizing*, *reducing* enzymes. This definition is not quite exact, since the urea fermenting enzyme is also a hydrolyzing enzyme, and the acetic fermentation is caused by an oxidizing enzyme. The distinction between *endo-enzymes* (*intra-cellular*) and *exo-enzymes* (*secreted*) is not exact, either, since invertase and lactase are retained in the cells of some organisms and secreted by others.

The following classification is used in the further discussions:

I. *Hydrolytic Enzymes.*
  1. of carbohydrates: cellulase (cytase), diastase (ptyalin, amylase), invertase, lactase, maltase.
  2. of fats: lipase (steapsin).
  3. of proteins:
    (*a*) proteolytic (proteases): pepsin (peptase), trypsin (tryptase), erepsin (ereptase).
    (*b*) coagulating (coagulases): thrombase, rennet (chymosin).

II. *Zymases.*
  1. of carbohydrates: alcoholase, lactacidase.
  2. of other nitrogen-free bodies: vinegar-oxidase.
  3. of proteins: endo-tryptase, autolytic enzymes, amidase, urease.

III. *Oxidizing Enzymes.*
  Vinegar-oxidase, tyrosinase.

IV. *Reducing Enzymes.*
  Katalase, reductases of nitrates, sulphur, sulphites, telluric salts, methylene blue, litmus.

Several different names have been given to some of the enzymes; these are found in parenthesis in the above classification.

The general action of enzymes being explained in the preceding pages, it remains to describe more in detail the different enzymes of microbial origin.

HYDROLYTIC ENZYMES

ENZYMES OF CARBOHYDRATES.—Enzymes which decompose carbohydrates are very commonly found in nature, because carbohydrates constitute a very extensive and common group of organic matter. By far the largest part of the dry plant consists of cellulose, starch and sugar. To decompose them, enzymes are necessary. The chemical reaction of these enzymes is hydrolytic; in other words, the larger molecule is broken into smaller ones by the simple addition of water. Thus, the cellulose-destroying enzyme, called *cellulase* or *cytase*, decomposes the cellulose into soluble sugars after the following formula:

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$$

or, considering that the cellulose molecule is really many times $C_6H_{10}O_5$, the formula will be more accurately written

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$

which indicates at the same time that one cellulose molecule gives many sugar molecules.

Cellulase is an enzyme which is quite difficult to obtain. Though it must be produced by all the cellulose destroying molds and bacteria, experiments have failed in some instances to prove its presence. It is found in some wood destroying fungi and in some of the bacteria causing the rot of vegetables. The organisms of certain plant diseases force their way into the cell by dissolving the cellulose membrane by an enzyme, while certain molds are able to puncture the cell wall mechanically.

Diastase, or amylase, is the starch-dissolving enzyme which is one of the most common enzymes in nature. It is found in all green plants, and it forms during the sprouting of starchy seeds. Many molds and a few bacteria produce this enzyme, while yeasts generally cannot decompose starch for lack of diastase. Starch has the same formula as cellulose, and it is broken up into soluble sugars in the same way. Much attention has been paid to this process by the chemists, and it is found that the process is a gradual one, giving first dextrins, and finally maltose ($C_{12}H_{22}O_{11}$). The hydrolysis of starch expressed in chemical symbols may be presented as follows:

$$\underset{\text{Starch}}{2(C_6H_{10}O_5)_n} + nH_2O = \underset{\text{Maltose}}{nC_{12}H_{22}O_{11}}.$$

The disaccharides or double sugars, having the chemical formula $C_{12}H_{22}O_{11}$ are broken up into single sugars, monosaccharides, by the following process:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

The two molecules of $C_6H_{12}O_6$ are different with different sugars. If the disaccharide is saccharose, the two monosaccharide molecules are dextrose and levulose. Lactose will yield dextrose and galactose, and maltose will give two molecules of dextrose. For each of these sugars, there is a special enzyme which can hydrolyze only its particular sugar and none of the others; like a key, made for one lock, it will not open another lock. *Maltase* will split only maltose molecules, not lactose, while the *lactase* cannot attack the maltose. *Invertase* (or *sucrase*) will decompose nothing but saccharose. This decomposition of the complex sugars into the simple sugars was believed to be necessary because only sugars of the type $C_6H_{12}O_6$ can be fermented directly by the fermenting enzyme in the cell, be it an alcoholic or lactic or gassy fermentation. This explains why beer yeast cannot ferment lactose; it produces no lactase, and therefore cannot attack the lactose molecules; they would be easily attacked, if besides the yeast, some lactase were added. Certain lactic bacteria cannot ferment saccharose, because they do not form invertase. Recent experiments have shown that bacteria exist which ferment lactose and saccharose but not dextrose or levulose. An explanation for this cannot be given.

Invertase is, like diastase, a very common enzyme in green plants. It is also produced by most molds and yeasts, and bacteria. Maltase is not quite so common, and lactase is limited to a few species of microörganisms. A few organisms are known which do not secrete these enzymes but retain them within the cell. This is especially true of lactase, but is also known, in a few instances, of invertase. The enzyme can be obtained from the broken cells. Such enzymes are called *endo-enzymes*.

The decomposition of carbohydrates has been followed from the most complex representatives to the simplest ones, the monosaccharides. If these are decomposed further, the resulting product is no longer a carbohydrate. The simplest sugars are decomposed by zymases, inside the microbial cell, into compounds which are generally called fermentation products; these may result from alcoholic, lactic, butyric fermentations or some other.

Emulsin is an enzyme which is able to hydrolyze glucosides. Glucosides occurring in plants are complex bodies which contain a sugar-radical. Emulsin splits glucosides liberating the sugar, usually dextrose. The typical example for emulsin action is the hydrolysis of amygdalin to hydrocyanic acid, benzaldehyde and dextrose.

$$\underset{\text{Amygdalin}}{C_{20}H_{27}O_{11}N} + 2H_2O = \underset{\text{Benzaldehyde}}{C_6H_5COH} + \underset{\text{Dextrose}}{2C_6H_{12}O_6} + \underset{\text{Hydrocyanic acid}}{HCN}.$$

Emulsin is found in many molds and bacteria, and recently has been found in yeasts. Glucoside-splitting enzymes play an important rôle in the fermentations of coffee-beans, cocoa, mustard and indigo. In most of these fermentations, however, the emulsin is probably not formed by microörganisms, but by the plant, from which the fermenting material is derived.

ENZYMES OF FATS.—All the enzymes, acting on fat, decompose it in the same manner; the fat molecule takes up three molecules of water, breaking up into glycerin and three molecules of fatty acid, as indicated on page 239. It is possible that there are several fat-splitting enzymes, but the result of the cleavage process is always the same. The name formerly assigned to enzymes of fat is *steapsin*, but this term is now almost exclusively substituted by the more significant word *lipase*. Occasionally they are called lipolytic enzymes which expression is analogous to the proteolytic enzymes; in the same way, the term amylolytic enzyme is used for diastase.

ENZYMES OF PROTEINS.—The enzymes composing protein bodies, generally called proteolytic enzymes or *proteases*, have been known for nearly a century. Though the difficulty of analyzing protein bodies accurately prevents an absolute knowledge of proteolysis, much effort has been made to become acquainted with the very important group of enzymes which accomplish the digestion of protein food. Naturally most experimenting has been conducted with pepsin and trypsin of the animal body and accordingly these are better understood than others; only little work has been done with microbial enzymes. There is so far as can be determined little appreciable difference between the proteolytic enzymes obtained from different organisms, whether low or high in the plant or animal world, consequently many experiences with animal pepsin and trypsin can be applied to microbial enzymes.

The specific chemical action of these enzymes is referable to hydrolysis; the large protein molecule is broken up into smaller molecules by addition of water. Various proteolytic enzymes differ in the extent of decomposition. While some, like pepsin, produce mainly peptones, trypsin is able to split protein to amino-acids and even to ammonia. Mavrojann is tested for the intensity of gelatin decomposition with formaldehyde. The peptones of gelatín will solidify with formaldehyde while amino-acids are not affected.

Proteolytic enzymes were first divided into two groups: *pepsins*, which act best in slightly acid solutions, and *trypsins*, which act best in slightly alkaline media. The names are derived from pepsin (peptase) the proteolytic enzyme of the animal stomach, and from trypsin (tryptase) which is found in the small intestine of animals. This classification cannot be used for the enzymes of microörganisms because there is no definite line established by the acidity. Some enzymes work in either acid or alkaline media equally well, preferring a neutral reaction. Enzymes should be classified according to the substances they act upon or perhaps according to the nature of the products resulting from the fermentation. This would bring pepsin and trypsin into one class, both acting upon protein bodies as such; they, however, differ in the intensity of action as shown by their products, the pepsin forming mainly peptones, the trypsin carrying on the decomposition as far as amino-acids and traces of ammonia. Another class recently recognized is *ereptase* (erepsin) which cannot decompose protein, but readily attacks peptones, decomposing them much in the same way as trypsin. Pepsin, trypsin and erepsin do not break up amino-compounds.

The presence of proteolytic enzymes in microörganisms is readily tested by cultivation on nutrient gelatin. The proteolytic enzyme secreted by the cells will liquefy the gelatin. Generally, an organism that liquefies the gelatin will also decompose the casein of milk and the protein of blood serum. There are some exceptions, however, as is shown in the following table, after Frost and McCampbell. A + sign means proteolysis, a − sign means no action.

Organism	Milk		Gelatin	Serum	Egg album.	Fibrin
	Coag.	Digest.				
Bact. anthracis	+	+	+	−	+	+
Microspira comma	+	+	+	+	+	+
M. pyogenes var. aureus	+	+	+	−	−	−
Pseudomonas pyocyanea	+	+	+	+	+	−
B. violaceus	−	−	+	−	−	−
B. mycoides	+	+	+	−	+	−
B. prodigiosus	−	+	+	+	+	+
Aspergillus niger	+	+	−	−	−	−
Aspergillus oryzæ	−	+	+	+	+	−

Apparently not all organisms which liquefy gelatin are able to decompose egg albumin; we must conclude that the enzyme liquefying gelatin is different from the proteolytic enzyme dissolving egg-white.

Coagulating Enzymes.—The blood-clotting enzyme (*thrombase*) does not occur in microörganisms. *Rennet*, however, is found in many species. Rennet is extracted from the stomach of calves and pigs and used to set the curd in milk for cheese making. The enzyme acts upon the casein in milk, decomposing it into paracasein and some soluble protein. The time of coagulation depends upon the temperature of the milk and the concentration of the rennet. This coagulation of milk is quite different from the acid curd, where the insoluble casein is precipitated by the acid. If enough acid is added, the milk curdles immediately; if there is not enough acid, there will be no curd, not even after a long time. An acid curd can be brought back to the original state by an addition of alkali, while a rennet curd by no means can be changed back to casein. Rennet-forming bacteria are found in milk and dairy products, in soil and other habitats. They will coagulate milk without causing any appreciable increase of acidity. They all seem to digest the curd after it is formed (see the above table). The relation between proteolytic and rennet enzymes will be discussed in a later chapter.

Rennet is sometimes called chymosin; the Society of American Bacteriologists uses the German word "*lab*."

ZYMASES

The zymases are the agents which furnish the energy for cell life by causing fermentative decompositions. As has been stated before, the processes which provide for energy must take place inside of the cell. Consequently, all fermenting enzymes are endo-enzymes. The difference between the soluble enzymes and the endo-enzymes is very plainly shown in the following table, giving the energy liberated by the various enzymes by acting upon 1 g. of substance.

ENERGY LIBERATED FROM 1 G. OF SUBSTANCE

Soluble Enzymes		Endo-enzymes	
Pepsin, trypsin	0 calories	Lactacidase	80 calories
Lipase	4 calories	Alcoholase	120 calories
Maltase, invertase	10 calories	Urease	230 calories
Lactase	23 calories	Vinegar-oxidase	2,500 calories

The microbial cell does not lose much energy by the activity of the soluble enzymes outside of the cell, because their energy yield is insignificant.

The first zymase known was *urease*, the enzyme which changes urea to ammonium carbonate. The actual investigation of the zymases did not start until Buchner had demonstrated that yeast can be ground with infusorial earth until all cells are lacerated, and then can be pressed and the juice filtered without losing the power of alcoholic fermentation. Such fermentation cannot be due to anything but a soluble compound of the yeast cell. Thus the *alcoholase* was discovered. It was found later that yeast may be killed by alcohol, ether or acetone without losing its fermenting power.

This last method was applied later to lactic bacteria, and it was proved that the lactic acid is also produced by an enzyme, *lactacidase*. It is possible to kill the lactic bacteria so that they do not multiply but still continue to form acid. It seems quite probable that other fermentations of carbohydrates, like the butyric and the gassy fermentations, are really due to enzymes. It is very difficult to give the experimental proof, however. These enzymes are so unstable that it requires much experience to separate them from the cell, and it is also quite difficult to obtain bacteria in quantities large enough for such experiments.

The vinegar oxidase is an enzyme which remains in the cell of the acetic bacterium, oxidizing alcohol to acetic acid. Its independence of the living cell has been demonstrated by killing the cells with acetone.

The PROTEOLYTIC ENDO-ENZYMES of yeasts, only, have been studied extensively. That such enzymes exist is recognized by the observation that certain microörganisms do not liquefy the gelatin until after they are dead and the proteolytic enzymes diffuse out through the deteriorating cell membranes. That yeast in the absence of sugar loses in weight, and that leucin and other cleavage-products of protein are formed, was the first indication of a proteolytic process in the yeast cells. By pressing the juice out of the ground yeast cells, a liquid is obtained which liquefies gelatin, digests casein, albumin and fibrin. The living yeast cell does not attack these compounds, because they cannot diffuse into the cell and the enzyme cannot diffuse out. The proteolytic endo-enzyme of yeast is called *endo-tryptase.* Its object is apparently the regulation of the protein-content of the cell and perhaps it has some bearing on the formation of cell plasma. The possible relation between enzymes and growth is discussed in a following sub-chapter.

If yeast is mixed with a weak antiseptic (chloroform, toluol) the proteolytic process takes place quite rapidly. This process is called *autolysis* (self-digestion). Similar autolytic enzymes are found in other microörganisms. Autolysis is a well-known process in the higher animals. To this is due the ripening of meat.

Proteolytic endo-enzymes must be expected in all microörganisms which depend upon protein as food material only. These organisms will secrete certain enzymes which decompose the insoluble protein into bodies which diffuse easily into the cell. Here, proteolytic endo-enzymes further decompose these products. Such an endo-enzyme is the *amidase* discovered by Shibata in the mycelium of *Aspergillus niger* which forms ammonia from urea, acetamid, oxamid, biuret. *Endo-erepsin* and amidase were also found in *Penicillium camemberti* by Dox.

Similar to these proteolytic enzymes is the *urease* which is formed in large quantities in the so-called urea bacteria, but it is also present in the mycelium of some molds. An endo-enzyme, splitting hippuric acid into benzoic acid and glycocoll, is found in the mycelium of a few molds.

OXIDIZING ENZYMES

The most typical example of an oxidizing enzyme is the *vinegar-oxidase,* because its chemical action is well known. Most of the *oxidases* known act upon complex organic compounds, changing them to colored bodies. Such an oxidase is the *tyrosinase* which forms a black, insoluble compound in tyrosin solutions. It is produced by several bacteria, especially by chromogens, and its application in testing for small quantities of tyrosin has been suggested. A number of oxidases are known to act upon the leuco-bodies of certain organic dye-compounds, as aloin, guaiac, phenolphthalein, and others. Hydrochinon is oxidized by the dead cells of a few molds. Strange seems the oxidation of potassium iodide to iodine by the *endo-oxidase* of a mold. Many other oxidations are supposed to be of enzymic nature, but their independence of the living cell has not been proved.

Many higher organisms are known to contain oxidases, the best studied are those of certain mushrooms which change the white mushroom meat into a bluish or brownish color as soon as it is exposed to the air. Oxidases are very common in most of the tissues of higher animals.

REDUCING ENZYMES

Among the *reductases,* one enzyme stands apart from all the others, that is the *katalase* or *peroxidase* which reduces the hydrogen peroxide to water by liberation of oxygen.

$$H_2O_2 + \text{katalase} = H_2O + O.$$

Katalase is one of the most commonly found enzymes; it is formed by practically all plants and all animals and is contained by all but a few bacteria. Among these exceptions is the *Strept. lacticus.* The absence of katalase in this species has been recommended as a diagnostic test. It is possible that this enzyme is necessary for intra-cellular oxidations.

A number of other *reductases* are known. Nearly all of the reductions mentioned in the paragraph on the products of mineral decomposition are proved to be of enzymic nature; these processes will take place after the cell is killed by a disinfectant or is ground to pieces. This can be readily demonstrated by lacerating the cells with quartz sand. They will then reduce nitrates to nitrites, sulphur to hydrogen sulphide. The decolorization of litmus, methylene blue, indigo, and other organic dyes is due in microbial cultures to enzymes which are almost exclusively endo-enzymes.

Enzymic Theory of Katabolism

Regarding katabolism as the sum of all destructive processes of the living cell substance, *i.e.*, of the protoplasm, and considering the cell substance to be decomposed and renewed constantly as long as the cell is performing the normal functions of life, there must be a renovating and a destructive process continuously going on in the protoplasmic molecules. If the food supply ceases, anabolism ceases with it, but it has been demonstrated that katabolism may continue just the same for some time. By this method, the products of katabolism can be obtained separate from the products of food digestion which would obscure the results of experiment on katabolism in normally fed cells.

It is difficult to determine to what extent katabolism is controlled by *endo-enzymes*, the so-called *autolytic enzymes*, which have been mentioned in the above paragraph. Unquestionably, the katabolic processes are similar to enzyme processes, since katabolism is checked by heat or poison just like enzyme processes.

Enzymic Theory of Anabolism

Anabolism and Intra-cellular Enzymes.—All changes discussed in the previous chapters are processes in which organic or inorganic compounds are broken up to smaller molecules. These processes are exothermic, *i.e.*, liberating heat or energy in other forms. The opposite is true of the anabolic processes which build up complex molecules from simple compounds. These synthetic processes are endothermic, absorbing heat or other energy. Growth is the typical manifestation of anabolism. It is the formation of new cells from dead organic or inorganic matter, and it means the formation of all the compounds necessary for cell life. Of all the substances found in the cell, practically none are contained in the food, and it is wonderful that in such a small unit as a microbial cell, there are contained the powers of making protoplasm, enzymes, nuclear bodies, chromatin bodies, the substance of the cell wall and probably many other unknown compounds. All these complex substances are generally made from simple food compounds as amino-acids, carbohydrates and others.

These synthetic processes of the cell will, like most endothermic processes, take place only if energy is provided. This condition is usually fulfilled in the living cell, due to the fermenting processes going on continuously. There is a strange interaction between anabolism and intra-cellular fermentation proceeding in the protoplasm and this linking together of destructive and constructive reaction is the basis of life processes. The life processes decompose certain substances, the energy liberated allows the formation of protoplasm, which again liberates energy. Thus a continuous formation of protoplasm is secured.

An explanation of anabolism based upon chemical experiments is not possible at the present time. In the study of intra-cellular destruction it is possible to trace most processes back to enzymic action. There our knowledge ceases because the nature and mode of action of enzymes is unknown. In the study of anabolism our knowledge has not even progressed so far. The most promising explanation at present is based upon the *reversibility* of enzymic action.

Reversibility of Enzymic Action

Chemical reactions between organic compounds proceed quite rapidly at first, then become slower and slower until the reaction stops entirely. The reaction is not complete at the time it reaches an equilibrium. If the equilibrium is disturbed by adding more of the reagents, the process will continue. If, however, the products of reaction are added, the reverse process will take place. Reactions between organic compounds can proceed either way, depending upon the relative concentrations of the reacting substances. The standard example is esterification. Acetic acid plus alcohol gives ester plus water,

$$\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Alcohol}}{CH_3CH_2OH} \rightleftarrows \underset{\text{Ester}}{CH_3COOCH_2CH_3} + H_2O.$$

The process goes to a certain equilibrium and stops. If ester is mixed with water, it gives acid plus alcohol, until the same equilibrium is reached. If acid and alcohol are added to a system in equilibrium, more ester will be formed. If ester is added, more alcohol and acetic acid will be formed. The same is true with enzymes, at least with some enzymes. Maltase will decompose maltose into two molecules of dextrose. In a concentrated solution of dextrose, however, maltase will form maltose, or a similar sugar, isomaltose. Lipase is able to produce fat from glycerin and fatty acids. A solution of albumose with trypsin or pepsin gives a precipitate of a body which is more complex than albumose and which gives the protein reactions. It is believed by many physiologists that pepsin and rennet are the same body. Under certain conditions, it has a dissolving power, under other conditions it has the power to coagulate.

The reversibility of enzymic action has given rise to much speculation about assimilation and growth. It seems reasonable to suppose that the cell forms its protoplasm from amino-acids by the reversed action of proteolytic enzymes. In the same way, cellulose may be formed from dextrose, fat from glycerin and fatty acids. Nearly all phases of growth can be accounted for in this way. This is nothing but theoretical speculation, and the only fact to support it is the reversibility of certain enzymes. The conditions under which chemical reactions take place inside of the cell are very largely unknown. There are so many processes going on at the same time that it is absolutely impossible at the present time to obtain a perfect understanding of all these reactions. Thus, our knowledge of growth is largely based upon analogy and speculation.

General Enzymic Considerations

Enzymes are produced only by living cells. After they are once formed, they act like chemical compounds, independent of the cell which produces them. Even the endo-enzymes follow only the law of enzyme-action and are not influenced by the cell which contains them. The enzymes are mostly influenced by their own products, and when a certain yeast ceases to ferment sugar at the concentration of 8.5 per cent of alcohol, this means that the alcoholase of this yeast cannot tolerate more than 8.5 per cent of alcohol. The inability of the cell to regulate enzymic action may account for the fact that often a culture produces an amount of fermentation products sufficient to kill all cells. This is observed in the lactic, acetic and alcoholic fermentations, and, perhaps, occurs in many others.

Probably all cells produce several enzymes Microörganisms feeding upon various foods must form various enzymes. Frequently several enzymes are necessary for the decomposition of one compound. *Rhizopus oryzæ* uses three enzymes in order to form alcohol from starch, first the diastase to change starch to maltose, then maltase to change maltose to dextrose and finally alcoholase to change dextrose to alcohol and carbon dioxide. The number of enzymes formed by certain microörganisms is surprising. *Aspergillus niger* has the reputation of forming almost all enzymes which have ever been found in microörganisms. *Penicillium camemberti* produces (after Dox) erepsin, nuclease, amidase, lipase, emulsin, amylase, inulase, raffinase, invertase, maltase and lactase. It has been believed for a long time that certain enzymes are regular products of the cell while others are formed only if the substance upon which they act is present. According to Dox's investigations with *Penicillium camemberti*, there is no evidence that enzymes not normally formed by the organism in demonstrable quantities can be developed by special methods of nutrition. The addition of a particular food compound does not develop an entirely new enzyme, but stimulates the production of the corresponding enzyme which is normally formed, although in small amounts, under all conditions.

CHAPTER III

FOOD OF MICROORGANISMS*

Moisture Requirement

Moisture may be called the most important factor of life. Not only bacteria, but every microscopic and macroscopic being requires a considerable amount of moisture. Living organisms contain on the average between 70 per cent and 90 per cent of water, and only 10 per cent to 30 per cent of solid matter. Microörganisms which live entirely submerged in liquids need water not only within but without the cells. Bacteria, yeasts, molds, and some protozoa obtain their food by diffusion through the cell-membrane; their food-substances must be soluble and dissolved. No other liquid can take the place of water.

The amount of water required by microörganisms cannot be stated briefly. Several factors have to be taken into consideration, as the osmotic pressure, the insoluble and the colloidal substances, the species of organisms, temperature, and perhaps others. (See pp. 184, 203.)

Amount of Food Required

The amount of food that is ordinarily decomposed by microörganisms and the amount that is absolutely necessary, differ widely. The quantity of organic and inorganic matter just sufficient to support a very weak growth is certainly very small, since a few species will multiply to some extent in ordinary distilled water. Such water, after having stood for some time, is found to contain several thousand bacteria per c.c. It may seem to the layman that in such water it would be possible to detect easily the organic and inorganic matter of the microörganisms so that it could not be considered distilled water. An estimate of the weight of bacteria demonstrates, however, that this is not the case. If we suppose the average bacterial cell to be a cylinder whose base measures 1 square micron and whose height is 2 microns (which is a high estimate) the volume of such a cell would be 1 × 1 × 2 cubic microns = 0.001 × 0.001 × 0.002 mm. = 0.000,-

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* Prepared by Otto Rahn.

000,002 cu. mm. The specific gravity of bacteria being very nearly 1, the weight of one bacterium would be 0.000,000,002 mg.; 100,000 cells per c.c. means 100,000,000 cells per liter, which would weigh 0.2 mg. Of this total weight, at least four-fifths is water and only one-fifth is solid matter. The total solid matter in 1 liter of water containing 100,000 bacteria per c.c. amounts to the immeasurable quantity of 0.04 mg. Such water will pass the tests for distilled water. How much food the bacteria in distilled water have used is impossible to say, since besides the traces of minerals in the water, they obtain some food from volatile compounds of the air like carbon monoxide (CO), carbon dioxide (CO_2), ammonia (NH_3), hydrogen (H), and perhaps methane (CH_4). Under all circumstances the amount of food used is very small.

On the other extreme, the maximum amount of food cannot be stated very definitely. Usually bacteria cease to cause decomposition because of the accumulation of noxious metabolic products. The ordinary bacterium from sour milk will not form more than about one per cent of lactic acid, because this is the highest acid concentration that this bacterium can endure. If this acid is neutralized, the inhibiting cause is removed, and the lactic fermentation starts anew until the maximum acidity is reached again. The amount of food decomposed depends largely upon the power of the organism to resist its own products. If the food is too concentrated, however, physical influences may interfere with the metabolism of the cell (page 254).

FOOD FOR GROWTH

The total weight of a large bacterial cell is estimated in the preceding paragraph to be about 0.000,000,002 mg., of which only about one-fifth is dry matter. The smallest quantity that can be weighed accurately on ordinary analytical balances is 0.1 mg. This corresponds to about 250,000,000 bacteria. MacNeal and associates found that the dry matter of 550,000,000 cells of *B. coli* weigh 0.1 mg. The amount of food that is used as the building material for the cell is probably larger than the weight of the cell itself, since there will always be present waste products, but it is of the same order of magnitude, *i.e.*, very small and often hardly measurable. The example of the urea fermentation (page 202) illustrates this point very well.

SOURCES OF CARBON.—The compounds which can serve as building stones for the cell vary greatly with the species. The source of carbon for all green plants is carbon dioxide (CO_2). Animals cannot use this, for they all require complex compounds, such as carbohydrates, fats or amino-acids. Bacteria exist between the plants and animals in this respect. Some bacteria have already been mentioned (page 201) as being able to use carbon dioxide (CO_2), as the only source of carbon; they are the mineral-oxidizing species. Such bacteria are called *autotrophic* in their relation to carbon, since they use it in the inorganic form. A bacterium feeding on carbon, as such, would be called *prototrophic;* bacteria of this class are said to exist. The vast majority of microörganisms are *heterotrophic*, using carbon in organic form. Organic acids and sugars are excellent sources of carbon for microörganisms, although proteins and their decomposition products seem to be equally satisfactory as construction material.

Sources of Nitrogen.—The sources of nitrogen are equally varied; the green plants use nitrates; animals must have a number of different amino-acids; the microörganisms again are found between plants and animals. We know *autotrophic* bacteria, and especially molds and yeasts which can grow with nitrates or ammonium salts as the only source of nitrogen. There are three groups of *prototrophic* bacteria in their relation to nitrogen—the *B. amylobacter* group, the *Ps. radicicola* group and the *Azotobacter* group. These bacteria are of the greatest importance to agriculture; soil fertility depends, to a large extent, upon the last two groups, for they take nitrogen gas from the surrounding air, form their own protoplasm from it, and thus increase the amount of chemically combined nitrogen in the soil. Details of their relation to soil fertility can be found in Chap. III, page 400. The majority of bacteria are *heterotrophic*, requiring organic nitrogen. Urea is not well adapted for this purpose; amino-acids or the peptones from which amino-acids are derived are the best compounds for most organisms. Asparagin is very commonly used if for some reason peptones are to be omitted.

Sources of Hydrogen and Oxygen.—The sources of hydrogen are hardly ever discussed with bacteria since hydrogen bears such a close and peculiar relation in water and organic food supplies. The ultimate association of hydrogen with oxygen in the molecule of water (H_2O) and with carbon in organic substances (CH_4) establishes its importance in all life processes. There are many *prototrophic* bacteria, using oxygen as such; others are able to reduce such compounds as nitrates or sulphates, which would be *autotrophic*, thus providing for their needs. *Heterotrophic* bacteria are not unusual. In this connection it may be said that it is often difficult to distinguish between oxygen needed for cell construction and oxygen needed for energy formation.

Sources of Minerals.—The amount of mineral matter necessary for the construction of the cell is very small; potassium and phosphorus seem to be among the most essential elements. It is customary to consider a tap water with 0.02 per cent of di-potassium hydrogen phosphate (K_2HPO_4), sufficient in mineral matter of all kinds to provide for fair growth. Some of the common materials used in the preparation of nutrient media, such as meat extract and peptone, also contain considerable amounts of mineral matter.

Food for Energy

As all food in its decomposition results in products of some form or other, it may not seem justifiable to separate a paragraph on *food* from another on *products*. The essential difference lies in the fact that we consider food from the viewpoint of the cell, while products are commonly considered apart from the construction processes of the cell and only from their application, or, it may be, from the viewpoint of usefulness to man.

Animals provide for their energy by oxidations, and almost exclusively by complete oxidations. Some bacteria, and most molds, do the same. The range of materials which can serve as food for this purpose is surprising. With animals, the food is practically limited to plant and animal tissue. With bacteria, we find the strangest substances, such as hydrogen, carbon monoxide, coal, marsh gas, hydrogen sulphide, ammonia, nitrites, formic and oxalic acids, alcohol and thiosulphates serving this purpose. The fact that many gases are used as food makes us realize that oxygen is not such an extraordinary compound as animal physiology seems to indicate, but that it should be classed merely as one of the many food compounds. This is especially significant since it will be shown later that free oxygen is not necessary for microbial life, and that many organisms can exist without it.

The oxidations are not always complete. The formation of nitrous acid from ammonia, the oxidation of alcohol to acetic acid are such examples. Some organisms are highly specialized in their food requirements, especially the mineral-attacking bacteria are usually limited to one source of energy. The microörganisms oxidizing organic compounds have, as a rule, the ability to decompose several compounds, and some bacteria are common scavengers, able to feed on organic acids, sugars, fats and proteins.

Oxygen Relations.—It is characteristic of many microörganisms to provide for their energy without using free oxygen. One such example has already been given in urea fermentation.

$$\underset{\text{Urea}}{(NH_2)_2\,CO} + 2H_2O = \underset{\text{Ammonium carbonate}}{(NH_4)_2CO_3}$$

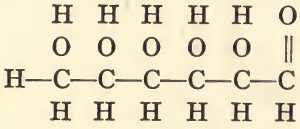
Very common is the decomposition of sugars without oxygen. The two most typical fermentations of this type are the alcoholic and the lactic fermentations.

$$\underset{\text{Sugar}}{C_6H_{12}O_6} = \underset{\text{Alcohol}}{2C_2H_5OH} + 2CO_2 + 22 \text{ Cal.}$$

$$\underset{\text{Sugar}}{C_6H_{12}O_6} = \underset{\text{Lactic acid}}{2C_3H_6O_3} + 15 \text{ Cal.}$$

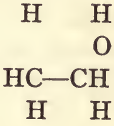
In fermentations of this type, the changes take place without an oxygen gas partaking in the reactions. These fermentations seem to be essentially reactions of the oxygen atoms within the sugar molecule. One side of the molecule is reduced while the other side is oxidized. In the sugar molecule, each carbon atom has one oxygen atom. In the products of fermentation, carbon dioxide has two oxygen atoms to one carbon atom, and in alcohol there is only one oxygen atom for two carbon atoms. In the lactic fermentation, the oxygen, which is distributed evenly in the sugar, is shifted to one side of the molecule in lactic acid.

Dextrose,



$$\begin{array}{ccccccccccccc}
 & & H & & H & & H & & H & & H & & O \\
 & & O & & O & & O & & O & & O & & \| \\
H & — & C & — & C & — & C & — & C & — & C & — & C \\
 & & H & & H & & H & & H & & H & & H
\end{array}$$

Alcohol,

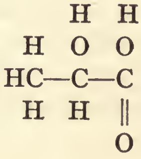


$$\begin{array}{ccc}
H & & H \\
 & & O \\
HC & — & CH \\
H & & H
\end{array}$$

$$\begin{array}{c}
O \\
\| \\
C \\
\| \\
O
\end{array}$$

Carbon dioxide,

Lactic acid,



$$\begin{array}{ccccc}
 & & H & & H \\
H & & O & & O \\
HC & — & C & — & C \\
H & & H & & \| \\
 & & & & O
\end{array}$$

In some of the more complex fermentations, we find simultaneous formation of hydrogen or methane and carbon dioxide; the one is the end product of reduction, the other the product of complete oxidation. This also indicates that the oxidation of one part of the molecule takes place at the expense of the other.

In a similar way, some organic acids, *e.g.*, tartaric and lactic acids, can be fermented by certain bacteria without requiring oxygen. Some bacteria have the ability to attack proteins and decompose them completely in the absence of oxygen.

Bacteria, having the ability to provide for their energy without oxygen gas, may live in the complete absence of oxygen, and may multiply indefinitely without it as long as there is sufficient food. But some microörganisms, such as yeasts, seem to grow only for a limited time in the absence of oxygen. Finally, they cease growing, and we may well assume that they need oxygen for cell construction which can be used in no other form except as molecular oxygen. The urea bacteria also belong in this group.

A large number of bacteria and yeasts, and also a few molds, can provide for their energy by either oxidation or decomposition in the absence of oxygen. Very commonly a great variety of compounds can be found which may be oxidized while but very few can be intramolecularly fermented without oxygen. This is easily understood: all organic compounds will yield heat upon oxidation, while exothermic intramolecular changes require a special structure. Carbohydrates are the most excellent substances for such intramolecular decompositions. *S. cerevisiæ* and *B. coli* can live in sugar-free broth only if exposed to the air. They provide for all their needs by oxidation of the protein. If oxygen is excluded, growth depends upon sugar, or a similar fermentable compound. We test for the absence of sugar in a given solution by pouring it in a fermentation tube and inoculating with *B. coli:* if the liquid in the closed arm remains clear, *i.e.*, if *B. coli* does not grow without oxygen, it is a good indication that no sugar is present.

It is usually assumed that in fermentations of this nature, the oxygen atoms are shifted within the same molecule. In other cases, oxygen is taken from one molecule and used for the oxidation of another. This results in one of the molecules being reduced. Nitrates are reduced in this way to nitrites, or ammonia, or nitrogen gas; sulphates to hydrogen sulphide, and litmus or methylene blue to the colorless leuco-compounds. Such removal of oxygen from a molecule requires energy, and is possible only when the bacterium by using the oxygen for oxidation of organic matter can obtain a larger amount of energy. The following example shows such a possibility:

$$2KNO_3 + 36.6 \text{ Cal.} = 2KNO_2 + O_2$$
$$C_2H_5OH + O_2 = CH_3CO_2H + H_2O + 115 \text{ Cal.}$$

This process leaves an energy balance of $115 - 36.6 = 78.4$ Cal. for the needs of the bacterium.

Such decompositions are sometimes referred to as "reducing fermentations" but this term is not correct, as the reduction must always be accompanied by a simultaneous oxidation process.

The amount of energy liberated by a fermentation without oxygen is much smaller than that furnished by complete oxidation; the intramolecular change always leaves organic compounds which contain a considerable amount of the total energy. Yeast, in presence of very much oxygen, oxidizes sugar completely to water and carbon dioxide.

$$C_6H_{12}O_6 + 12O = 6CO_2 + 6H_2O + 674 \text{ Cal.}$$

while in the absence of oxygen it will change the sugar to alcohol and carbon dioxide.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 22 \text{ Cal.}$$

The energy gained in the first process is about thirty times as large as that gained in the second process. This was demonstrated as early as 1861 by Pasteur. He grew yeast in sugar solutions, varying only the amount of oxygen in contact with the medium. At the end of the experiment, the weight of the dry yeast and the decomposed sugar was determined, and the amount of sugar necessary to produce one part of yeast was computed. He found:

In a closed flask, without any air..........	1 part yeast required	176 parts sugar.
In a closed flask, with large air space.......	1 part yeast required	23 parts sugar.
In a thin layer, a few mm. thick...........	1 part yeast required	8 parts sugar.
In a very thin layer, in 24 hours...........	1 part yeast required	4 parts sugar.

This experience led Pasteur to the conclusion that fermentation corresponded to the respiration process of animals, that fermentation was respiration without oxygen.

It is quite evident that since the utilization of the food in the absence of oxygen is very high, the organisms have to decompose much more food. This accounts, to a great extent, for the enormous destructive power of bacteria, when comparisons of the great quantity of food decomposed are made with the very insignificant weights of cells. It has been estimated that the lactic bacteria decompose their own weight of sugar in one hour.

Summing up the relation of oxygen to microörganisms, some bacteria, and especially the molds, are found depending upon oxygen as an indispensable part of their food. Three groups are recognized: Those, a large number, organisms in the presence of oxygen producing oxidations; those able to sustain life without oxygen; and those depending entirely upon decompositions which require no oxygen. The lactic bacteria and the butyric bacteria belong in the last group.

In considering the oxygen requirements, it is customary to include another influence of oxygen upon bacteria. This has really nothing to do with its food value, but deals with the poisonous qualities of oxygen. Oxygen in this light may well be called a poison as it will kill bacteria in very low concentrations. Ordinarily it is regarded as constituting over 20 per cent of our atmosphere. But if a study is made of its effect upon bacteria, it is necessary to measure it in the same way food is measured, and consider the concentration in which it is offered to the cell. Microörganisms obtain their oxygen not as gas, but as dissolved oxygen. The solubility of oxygen is very small, about 0.0009 per cent at 20°. Practically all bacteria die readily if the oxygen concentration is raised to thirty times the atmospheric pressure. This would mean a concentration of 0.027 per cent. It shows that oxygen is about as poisonous as formaldehyde or bichloride of mercury.

Some bacteria are extremely sensitive to oxygen, and will die if exposed to ordinary atmospheric oxygen. They grow only if oxygen is almost completely removed. These organisms are called the *strictly anaerobic* or *obligate anaerobic* bacteria. They are contrasted with the *facultative anaerobic* bacteria which thrive with oxygen as well as without, and the *strictly aerobic* bacteria which have to have oxygen for their normal life processes.

No strict limits can be drawn between aerobic and anaerobic bacteria. Even the most sensitive of organisms will be able to tolerate traces of oxygen, while the strictly aerobic bacteria can multiply also if the oxygen concentration is below that of a saturated solution. The limits of growth for the anaerobic bacteria are the limits of tolerance of the poisoning oxygen; the lower limit of growth for the aerobic bacteria is a question of too scanty food supply. The relation between bacteria and oxygen is graphically represented in the following diagram, after Kruse:

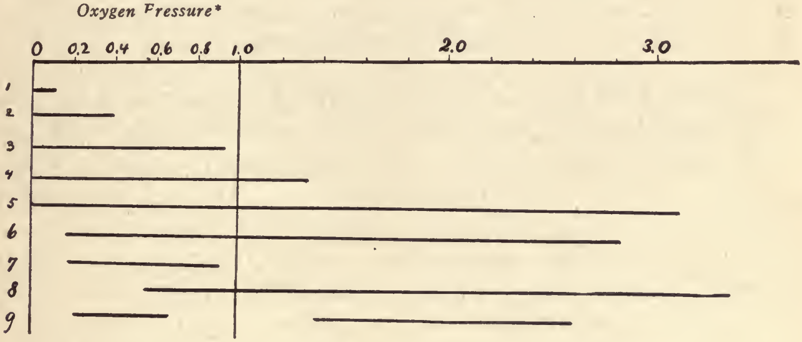


FIG. 112.—Influence of oxygen upon microörganisms.

The lines indicate the oxygen concentrations where growth is possible. Line 1 is a strict anaerobe; 2 is not quite so strict; 3 is still less sensitive though it cannot grow if exposed to direct influence of the atmosphere; 4 is a facultative bacterium such as *B. coli;* 5 is another one which can tolerate still more oxygen; 6 can grow only with oxygen but can get along with very little: it might be one of the urea bacteria; 8 is more dependent upon oxygen and the line would correspond to average molds; 7 is a peculiar type needing oxygen and yet being very sensitive to it. The sulphur bacteria, *e.g.*, the *Beggiatoaceæ,* belong to type 7. Type 9 is said to be representative of *B. abortus.*

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* 1.0 indicates the normal atmospheric oxygen content (about 21 per cent by volume).

CHAPTER IV

PRODUCTS OF MICROBIAL ACTIVITIES*

GENERAL CONSIDERATIONS

The great difference in the metabolism of animals and of bacteria, even though they feed essentially on the same foods, is the incomplete metabolism of most bacteria, contrasting sharply against the very complete oxidation of food in the animal body. The food of the animal is decomposed by the body cells to carbon dioxide, water and urea. It is the most complete decomposition possible, excepting urea which, however, is very near the final decomposition product, ammonium carbonate. Microörganisms, on the contrary, are characterized by incomplete metabolism. They do not commonly oxidize their food to the end products but many of them produce organic compounds which are not farther decomposed by them. It is this partial decomposition of organic matter which makes microörganisms play such an important rôle in life and industries. Our modern microbiology is dated from the time when Pasteur showed that the alcohol in the beer fermentation, the lactic acid in the souring of milk, the acetic acid in the vinegar fermentation are products of microbial activity. The existence of microörganisms had been known for nearly 200 years, but they were considered largely as a curiosity; as soon as they were recognized as the cause of fermentations, and of toxins, they received at once the greatest attention. Not all bacteria cause incomplete decompositions; some oxidize as completely as animals do. Others, again, form first intermediary products, which they later decompose completely; among these, are found many molds, the sulphur bacteria, and some species of the vinegar bacteria.

THE CHEMICAL EQUATIONS OF FERMENTATIONS

The metabolism of all organisms is considered to be a chemical process which follows in most respects the laws of chemistry. That we are not familiar with all the changes taking place in the cell is not because we are dealing with unknown forces, but simply because we do not know all the factors involved in the process. Some of the chemical changes caused by the living cell can be imitated exactly by the chemist in a test-tube. This may be illustrated by the oxidation of alcohol to acetic acid, the decomposition of urea to ammonium carbonate and of ammonia to nitrate. Some other processes are not as fully understood and not as easily imitated. The alcoholic and acid fermentations of sugars are of such nature. There is no reason to suppose, however, that these processes are other than chemical changes. Since a chemical process can always be expressed by a chemical equation, we should expect the same with the fermentations and decompositions caused by microörganisms.

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* Prepared by Otto Rahn.

This formulation is not always simple, because the greater number of microörganisms decompose organic substances in more than one way. Also, certain compounds may be produced in such small quantities as to escape the chemical analysis entirely, since the determination of many organic compounds is a very difficult task. Again, part of the decomposed material will usually be assimilated in the growth of the cells; hence more material disappears than can be accounted for by the fermentation products. There are several possibilities for discrepancies; accurate equations can be given only for the simplest fermentations, the products of which can be analyzed more or less exactly.

The best studied microbial process is the alcoholic fermentation. The simplest equation for the decomposition of dextrose into alcohol and carbon dioxide by yeast is

$$\underset{180}{C_6H_{12}O_6} = \underset{92}{2C_2H_5OH} + \underset{88}{2CO_2}$$

According to this formula, 100 parts of dextrose should give 51.11 parts of alcohol and 48.89 parts of carbon dioxide. The actual yield comes very close to these numbers, but does not reach them; the largest amounts found were 46–47.5 per cent of carbon dioxide and 47.5–48.67 per cent of alcohol. Under the most favorable conditions, the total yield of the products of fermentation was only 95 per cent of the theoretical yield.

Other products are formed besides the alcohol and carbon dioxide. The amount of glycerin found in fermented liquids varies very much with the conditions of fermentation; it reaches from 1.6 to 13.8 per cent of the alcohol or from 0.8 to 6.9 per cent of the fermented sugar. A small quantity of succinic acid is also formed, usually about 0.6 to 0.7 per cent of the fermented sugar. Traces of acetic acid and of lactic acid seem to be normal products of the process of fermentation, and we always find fusel oil. The latest investigations seem to indicate that glycerin and succinic acid are produced by yeast cells even in the absence of sugar. This discovery makes it probable that the glycerin and succinic acid are derived from the reserve substances of the yeast cells, such as lecithin, and are not direct products of fermentation. This accounts also for the variation of the proportion between alcohol and glycerin. Fusel oil is now believed to be a waste product of cell construction.

Similar are the experiences with the lactic fermentation which has been studied almost as extensively as alcoholic fermentation. If it is supposed that the formation of lactic acid follows the equation

$$\underset{\substack{342\\\text{Lactose}}}{C_{12}H_{22}O_{11}} + \underset{18}{H_2O} = \underset{\substack{360\\\text{Lactic acid}}}{4C_3H_6O_3}$$

the actual yield of acid is found to be between 90 per cent and 98 per cent of the theoretical. The other 2–10 per cent are either used for cell-growth or for products which thus far have escaped chemical determination. Small discrepancies will also be found in fermentation of urea and in the nitrifying process, where small amounts of the nitrogenous material are used for cell-growth.

Another difficulty in finding the chemical equation of a microbial fermentation is the fact that this process may change with the age of the culture. In those fermentations where several gases, as carbon dioxide and hydrogen, are produced, the relative proportion of the two is not always constant. In the butyric fermentation of dextrose by *B. amylozyma*, Perdrix tries to account for this change by assuming three different phases of the process at various ages of the cultures, represented by the following equations:

First stage: $\underset{\text{Dextrose}}{56C_6H_{12}O_6} + 42H_2O = 116H_2 + 114CO_2 + \underset{\text{Acetic acid}}{30CH_3COOH} + \underset{\text{Butyric acid}}{36CH_3CH_2CH_2COOH}$.

Second stage: $46C_6H_{12}O_6 + 18H_2O = 112H_2 + 94CO_2 + 15CH_3COOH + 38CH_3CH_2CH_2COOH$.

Third stage: $C_6H_{12}O_6 = 2H_2 + 2CO_2 + CH_3CH_2CH_2COOH$.

Kruse has called attention to the fact that these complex equations can well be explained as the simultaneous occurrence of the following simple fermentations:

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + CH_3CH_2CH_2CO_2H$$
$$C_6H_{12}O_6 = 3CH_3CO_2H$$
$$C_6H_{12}O_6 + 6H_2O = 6CO_2 + 12H_2$$

The first fermentation continues when the others have already ceased, and thus the last stage of Perdrix's equations is very simple. Bredemann also found that the proportion of the various products formed by *B. amylobacter* varies greatly with the conditions, and the same has been recently established in the fermentation of *B. coli.*

Other complications occur when an organism is able to use its own products as food, as is the case with some acetic bacteria. They will at first produce considerable amounts of acetic acid and after a while they oxidize the acid completely. It becomes impossible to account for microbial activity by a chemical equation when several organic compounds are decomposed at the same time as is found to occur in some foods, as butter, cheese, ensilage and in sewage. It is also impossible to formulate exactly decompositions which are caused by mixed cultures. The complications become so great and the relations between different organisms are so little known that it is useless to make the attempt.

PRODUCTS FROM NITROGEN-FREE COMPOUNDS

SUGARS.—It would be entirely beyond the limits of this book to give an account of all the different ways in which sugars and other compounds can be decomposed by microörganisms. It is much more important for the beginning microbiologist to acquaint himself with the main types of sugar fermentations and with the characteristics of the organisms which bring about these changes.

In the action of microörganisms many distinguish somewhat crudely six common types:

Complete oxidation.
Partial oxidation.
Alcoholic fermentation.
Lactic fermentation.
Acid gas fermentation.
Butyric fermentation.

Most of these types have been mentioned previously.

Complete oxidation of carbohydrates is observed most commonly among molds and mycodermas, and also in a few bacteria, *e.g.*, in *Azotobacter.* It is possible only where there is a ready oxygen supply, as, *e.g.*, in soils of an open texture, in trickling filters, and on the surface of decaying fruits.

The *incomplete oxidation* is, as a rule, more common in nature. Frequently microörganisms produce first an incomplete oxidation, but later oxidize the intermediate products completely. The molds are typical examples. *Aspergillus niger* is noted for its formation of oxalic acid. If it is grown in a sugar solution, it will bring about at first a rapid increase in acidity, but after a while, it decreases again, when the acid is oxidizing completely. The following processes may be noted:

$$C_6H_{12}O_6 + 9O = \underset{\text{Oxalic acid}}{3(CO_2H)_2} + 3H_2O$$
$$(CO_2H)_2 + O = 2\,CO_2 + H_2O$$

The intermediate product can be accumulated by precipitating it with lime which neutralizes the acidity. This principle is used in the commercial manufacture of citric acid by *Citromyces*, a mold closely related to the genus *Penicillium.* This mold oxidizes sugar to citric acid according to the following equation:

$$C_6H_{12}O_6 + 3O = \underset{\text{Citric acid}}{C_6H_8O_7} + 2H_2O$$

This fermentation is much more complicated than this equation indicates, on account of the entirely different chemical structures of citric acid and dextrose. The practical yield in the factory is only about one-half of the theoretical, since complete oxidation cannot be avoided altogether.

The oxidation processes, just recited, can take place only in the presence of oxygen; the other four types of carbohydrate decomposition require no oxygen, and take place as well in the absence of oxygen; the butyric fermentation is brought about only in the absence of oxygen.

Alcoholic fermentation is caused only by yeasts and a few molds; no bacterium produces alcohol according to the well-known equation mentioned above. Alcohol is formed by several bacteria but only in small quantities and always together with several acids; this is a distinctly different type of decomposition.

In the above groups and the following groups of microörganisms, there appears to be a close agreement between the morphological characters of the organisms involved and the specific type of fermentation. Practically all the alcoholic organisms are yeasts, and the lactic acid-producing organisms are streptococci or closely related bacteria.

The lactic bacteria, as they are briefly named, such as are responsible for *lactic fermentation*, are readily recognized by their scanty growth on agar, and their excellent growth in milk, bringing about a solid curdling in one to three days. They change sugar to lactic acid only.

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

No gas and no volatile acids are formed by these bacteria. The best-known representative of this group is the organism which causes the normal souring of milk. It was originally called *Bacterium lactis acidi*, but on account of its very close relation to the streptococci, it is more commonly now named *Streptococcus lacticus*. Many streptococci will produce the true lactic fermentation.

The last two groups of organisms, alcoholic and lactic, represent complex fermentations. There are several products formed, and as has already been pointed out in the paragraph on the equation of fermentations, the entire fermentation cannot be described accurately by one equation, for different fermentations operate independently and simultaneously in the same cell. Under slightly different experimental conditions the one or other of these simultaneous fermentations may be favored, accordingly a varying proportion of the products is formed.

The typical representatives of the acid-gas forming group of microorganisms which cause *acid-gas fermentation* are *B. coli*, and its near relative, *Bact. aerogenes*. Many of the gas-formers in nature belong in this group; the bacteria of the fermentations of pickles, sauerkraut, salt-rising bread, the gassy fermentation of milk are some of the many representatives. They are distinct rods, with good surface growth, and do not liquefy gelatin. They are commonly spoken of as the coli-aerogenes group. Some of them have peritrichate flagella, while others are not motile.

The fermentation of dextrose brought about by these organisms has been described originally by Harden in the equation:

$$\underset{\text{Dextrose}}{2C_6H_{12}O_6} + H_2O = \underset{\text{Lactic acid}}{2C_3H_6O_3} + \underset{\text{Acetic acid}}{CH_3CO_2H} + \underset{\text{Alcohol}}{C_2H_5OH} + 2CO_2 + 2H_2$$

Harden himself stated later that this equation holds only for one strain, and that we have several different strains distinguished by a proportion of products quite different from the one suggested by the equation. Recently Kamm has shown that a good mineral food (probably phosphates are the essential agent) favors a formation of gas and of volatile acids, while a scant supply of minerals causes the bacteria to produce mainly lactic acid. We must assume, therefore, at least two simultaneous independent fermentations:

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

and

$$C_6H_{12}O_6 + H_2O = CH_3CO_2H + CH_3CH_2OH + 2CO_2 + 2H_2$$

The first equation is already known to us; it is the true lactic fermentation. The second equation may be divided still further into several simpler equations.

B. typhosus, causing typhoid fever, is closely related to *B. coli*, but does not form gas. It forms, however, formic acid, HCO_2H, which, if decomposed, would give $H_2 + CO_2$.

The last type of sugar fermentations is the *butyric fermentation*, in which butyric acid is the most conspicuous, but not the only fermentation product. Acetic acid, hydrogen and carbon dioxide, and, with some organisms at least, ethyl and butyl alcohols are formed along with butyric acid. As already mentioned in the paragraph on the equation of fermentation, Kruse believes this fermentation to consist of several simultaneous fermentations, of which the most interesting at this stage is the one showing the formation of butyric acid.

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + C_4H_8O_2$$

The organisms producing butyric acid are mostly strictly anaerobic spore formers with a tendency to form spindle-shaped cells; they stain bluish-black with iodine and Bredemann gave the clostridium group one species name, *B. amylobacter*, as he found no distinct and characteristic differences between the many strains which he studied. Many members of this group have the ability to fix nitrogen, *i.e.* to build up their protoplasm without using any sources of nitrogen other than nitrogen gas. Most of the so-called "*Clostridium*" species belong in this group. Butyric acid is also formed by *B. tetani* and by *B. botulinus*, the latter of which causes the most dangerous kind of meat poisoning.

Of other sugar fermentations may be mentioned here only by name, the slimy fermentations, as manifested in ropy milk and the mannit fermentation. The latter is one of the very few reduction processes brought about by bacteria, and one which causes trouble in wine.

What has been stated broadly for sugars holds to some extent true also for the alcohols derived from sugars, including glycerin. Many bacteria fermenting dextrose can also ferment mannit and glycerin with a slight variation of the products, but some do not do this.

Among disaccharides there is a great variation of fermentation. Some groups ferment lactose readily as the coli organisms and *Strept. lacticus*, while among yeasts, fermentation of lactose is rare. Practically all yeasts ferment saccharose, however, and among the lactic bacteria and the coli group many strains cannot ferment saccharose.

STARCH.—Quite different is the fermentation of the insoluble carbohydrates of which we can mention only starch and cellulose. Insoluble compounds can be fermented only after being made soluble by an enzyme, the amylase (see mechanism of metabolism). Amylase is produced by most molds, by none of the fermenting yeasts, by a few torulas, and perhaps mycodermas, and by a great many of the bacteria. The sugar thus produced from starch is decomposed according to the main types mentioned under sugars. The lactic bacteria and the coli bacteria do not attack starch, but some acid-gas fermentations of starchy foods do take place. Butyric fermentation of starch is common. Alcoholic fermentation can be accomplished only by some of the *Mucors*, and *Aspergilli*.

CELLULOSE is decomposed only by very few organisms; these must be very active and very numerous, to judge from the enormous amounts of cellulose produced and destroyed every year on earth. Molds and higher fungi play probably the main rôle in its decomposition; the products have not been determined, but we may well assume a complete oxidation, since no intermediate products have ever been mentioned. No yeast is known to decompose cellulose, and among the bacteria we find but very few species. Some species have recently been isolated which decompose cellulose in the presence of air; the products have not been determined; we can, however, assume a partial oxidation, eventually a complete oxidation. Besides the aerobic fermentation, we have two types of anaerobic fermentation which are ordinarily described as the hydrogen fermentation and the methane fermentation. In these fermentations the gases mentioned, together with carbon dioxide, are liberated, and butyric and acetic acids are formed at the same time. The marsh gas of the marshes originates in this way.

Summing up all the products formed from carbohydrates, we find several acids, among them lactic and acetic acids most commonly, and ethyl alcohol, rarely other alcohols, besides carbon dioxide, hydrogen and water. The variety is not so great, but with these few compounds, a number of different combinations are possible, and the complication of the study of such fermentations lies mostly in the simultaneous formation of several of the compounds.

Acids and Alcohols.—The organic acids and alcohols can be decomposed further by bacteria and molds, also by some yeasts, to simpler compounds. Ordinarily, this decomposition consists in the complete oxidation. Thus, *Oidium lactis* will destroy the lactic acid of sour milk and of soft cheeses by complete combustion.

$$C_3H_6O_3 + 6O = 3CO_2 + 3H_2O$$

By the same process, the acidity of sauerkraut, ensilage, pickles is reduced by mycoderma species. Another *Mycoderma* is known to destroy acetic acid and thus spoil vinegar or fruits and vegetables kept in vinegar; the yeast grows in a thin, dry white scum over the surface, and oxidizes the acetic acid.

$$CH_3CO_2H + 4O = 2CO_2 + 2H_2O$$

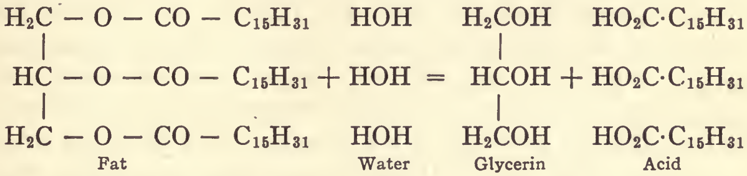
The oxidation of alcohols is not always complete. Especially ethyl alcohol is usually oxidized first to acetic acid; this is the common vinegar fermentation. Many different kinds of vinegar bacteria are known, some forming gelatinous masses of cell membranes called mother-of-vinegar, while others remain as separate small cells. They all oxidize alcohol first to acetic acid.

$$CH_3CH_2OH + 2O = CH_3CO_2H + H_2O$$

But most of them will oxidize later the acetic acid completely to carbon dioxide, after the alcohol is all exhausted, unless the oxygen supply is shut off. This behavior reminds one of the formation and destruction of oxalic acid by *Aspergillus*, mentioned previously. It may be remarked here that the vinegar bacteria cannot attack the sugar directly to any appreciable degree, and the manufacture of vinegar from sugar requires two agents, the alcohol-forming yeast, and the alcohol-oxidizing bacterium.

Some of the acids can also undergo an anaerobic fermentation. This is possible only with hydroxy-acids. The fermentation of the calcium salt of tartaric acid was the first anaerobic fermentation observed by Pasteur, and the fermentation of lactic acid to butyric acid has a reputation for its chemical peculiarity. A compound with four carbon atoms is formed from a compound with only three carbons, a very unusual thing in fermentation.

FATS.—The decomposition of fats is comparatively simple. All fats are glycerides of organic acids, and if they are attacked at all by microorganisms, they are first split into glycerin and free acid.



$$\begin{array}{lcccccc}
H_2C-O-CO-C_{15}H_{31} & & HOH & & H_2COH & & HO_2C\cdot C_{15}H_{31}\\
| & & & & | & & \\
HC-O-CO-C_{15}H_{31} & + & HOH & = & HCOH & + & HO_2C\cdot C_{15}H_{31}\\
| & & & & | & & \\
H_2C-O-CO-C_{15}H_{31} & & HOH & & H_2COH & & HO_2C\cdot C_{15}H_{31}\\
\text{Fat} & & \text{Water} & & \text{Glycerin} & & \text{Acid}
\end{array}$$

This brings about the liberation of three molecules of free acid from a neutral fat molecule. It is customary to test for the splitting of fat by determining its acidity. The glycerin is readily used up by the microorganisms, while the fatty acids are oxidized but very slowly.

The number of organisms which can attack fat is quite small. Most molds can destroy it; one torula has been found in butter which attacks it, and perhaps a dozen species of bacteria will do the same, among them *B. fluorescens* and *B. prodigiosus*, which cause occasionally the rancidity of butter.

PRODUCTS FROM NITROGENOUS COMPOUNDS

On account of the complexity of the protein molecule, the products of protein decomposition by microörganisms are little known. Some products are conspicuous through their odor, others can be told by certain color reactions, but as we cannot, at the present, give the structural formula of proteins, there is no possibility of stating protein decompositions in equations similar to those of carbohydrate fermentations. The discussion must be limited, for this reason, to the enumeration of the most important products, and to the general types of decomposition.

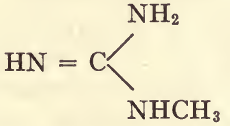
As in the carbohydrates, soluble compounds are more easily decomposed than the insoluble. The keratin bodies of hair, epidermis and horn are slowly attacked by a very few organisms. Gelatin, casein and serum albumin are more readily decomposed, though their solubility is quite limited. Peptones which are readily soluble are used by the vast majority of microörganisms. Of interest in this connection is the fact that the fresh white of egg is poisonous to most bacteria, and fresh blood and animal tissues as well as freshly drawn milk have also germicidal properties which are lost by heating or upon standing.

Protein bodies are as numerous as plants and animals. Each species of organism seems to have its particular protein which differs from that of other species. With the more highly developed organisms, there are several distinctly different proteins found in the same individual in different parts of the body. The constituents, carbon, oxygen, hydrogen, nitrogen, and sometimes sulphur and phosphorus can be determined in their relative amounts without, however, furnishing any knowledge of the structure of the molecule. The molecular weight of proteins is estimated to be at least 10,000, while the weight of the very large molecule of saccharose is only 342. The protein molecule can be broken up into smaller molecules. This cleavage is generally believed to be a hydrolytic process similar to the decomposition of starch to maltose. The first products of protein decomposition do not differ essentially from the original protein, but they can be hydrolyzed again and again, until finally products of a crystalline nature are found which are well-defined chemical bodies. Among the very first products of protein degradation it is usually impossible to determine single compounds, but several groups of compounds may be separated by certain precipitants, as acetic acid, ammonium sulphate, zinc sulphate, copper sulphate, tannic acid and others. In order to determine the degree of protein degradation, *e.g.*, in the analysis of cheese, it is customary to determine the nitrogen of compounds precipitated by these various reagents, and state it in percentage of the total nitrogen. Thus the terms "water-soluble nitrogen," "acid-soluble nitrogen" and others originated, meaning the nitrogen of the compounds soluble in water or in acid respectively. Some of these groups of degradation products have been named and defined more accurately, of which the albumoses and peptones are the most common and best described compounds. Their chemical nature and structure is, however, just as little known as that of the protein bodies. We speak of peptonisation of proteins, *e.g.*, in the clearing of milk or the gelatin liquefaction, meaning that the insoluble protein has been made soluble.

The amino-acids are the first well known compounds of protein decomposition. They are organic acids, in which a hydrogen atom is substituted by a NH_2 radical. Some of them are simple compounds, as the amino-acetic acid NH_2CH_2COOH and also the amino-capronic acid usually called leucin $(CH_3)_2CH\ CH_2\ CH(NH_2)\ COOH$. Others are of a more complex nature, such as the tyrosin or hydroxy-phenyl-aminopropionic acid, $C_6H_4(OH)\ CH_2CH\ (NH_2)\ COOH$, and the tryptophan or indol-amino-propionic acid, $C_8H_6N\ CH_2CH(NH_2)\ COOH$.

Of other nitrogenous products which are not amino-acids, a few are of striking significance. The very disagreeable odor of putrefying proteins and of excreta is due to indol (C_8H_7N) and methyl-indol or skatol ($C_8H_6N{\cdot}CH_3$). Indol gives a rose color with nitrites in acid solution, and this convenient reagent is used in the identification of bacteria. Another group are the amins. The simplest amins are the methyl-amins, of which the tri-methylamin $(CH_3)_3N$ is produced by several bacteria. The fishy odor of the brine of salted herring is largely due to this compound. In this group belong also a large number of the so-called *ptomains*.

The ptomains (page 592) are alkaloid-like bodies of basic character and of more or less well-known structure. Some of them are notorious for being very strong poisons, while others are quite harmless. These bodies are called ptomains because they were first discovered in putrefying corpses. The best-known compounds of this character are the putrescin or tetra-methylen diamin $[NH_2(CH_2)_4NH_2]$ and the cadaverin or penta-methylen-diamin $[NH_2(CH_2)_5NH_2]$, which can scarcely be considered poisonous. The methyl-guanidin



$$HN = C\begin{cases} NH_2 \\ NHCH_3 \end{cases}$$

may be mentioned as an example of a very poisonous ptomain. Another poisonous ptomain is the neurin $CH_2 = CH - N(CH_3)_3OH$ which has been found frequently as a product of putrefaction.

Ammonia is the end product of protein decomposition, as far as the nitrogen-containing fragments of the protein molecule are concerned. That ammonia is formed by many microörganisms, is well known. In some decaying proteins, *e.g.*, in old Camembert cheese, ammonia can be very easily detected by the smell. As all proteins contain many amino-groups as well as acid-amid groups, it is easily understood how the ammonia originates through the hydrolysis of protein. In the complete oxidation of proteins the nitrogen is always left as NH_3 or $(NH_4)_2CO_3$, respectively, never, so far as known, in any other form. No bacterium is known to produce urea, as most of the higher animals do.

In the products of protein degradation mentioned above only those compounds have been considered which contain nitrogen. It is quite evident, however, that in the cleavage of the large and complex protein molecules, certain parts of the molecule will contain no nitrogen. Many organic acids, like acetic, butyric, capronic, benzoic and phenylacetic acids are quite generally found among the products of putrefaction. Alcohols too, especially benzene derivatives like phenol and cresol, are not unusual. Gas is often formed in putrefaction, especially carbon dioxide and hydrogen; occasionally these gases are mixed with traces of nitrogen and methane.

Many protein compounds contain, besides the organic elements, larger or smaller amounts of phosphorus and sulphur. The phosphorus compounds may be changed to phosphine (PH_3), which is a gas of a strong disagreeable garlic odor. Generally, however, the phosphorus of protein after its degradation is found as phosphoric acid (H_3PO_4). Very little is known about the phosphorus of organic compounds and the changes it may undergo in the putrefactive process.

The sulphur of proteins is commonly changed to hydrogen sulphide (H_2S). Some microörganisms are able to form mercaptan (CH_3SH), a compound of very foul penetrating odor.

After this enumeration of the products, the main types may be considered briefly; since much less work has been done on protein decomposition than on carbohydrate decomposition, the groups are not so well defined. We might consider the following types:

Complete Oxidation.—This is brought about by many molds, by yeasts if they depend upon proteins only, and by many bacteria, of which the large, aerobic spore-forming rods, such as *B. mycoides*, are the main representatives. The products of oxidation are CO_2, H_2O,

NH_3 and H_2SO_4. The nitrogen is never changed to any oxidation product, but is found as NH_3, while the sulphur is oxidized.

Incomplete oxidation is caused by other bacteria, and perhaps molds and yeasts. Quite a large number of organisms live on sugar-free media if they have oxygen, but they do not oxidize their food completely. We can distinguish at least three different groups of microorganisms here.

B. proteus is the collective name for a number of closely related forms which belong to the most common organisms found on decaying organic matter, especially when protein is abundant. They produce leucin, tyrosin and tryptophane, but no skatol, or phenol. Indol and hydrogen sulphide are formed in certain media. Less important, but also very common are the pigment-forming rods among which *B. fluorescens*, *B. prodigiosus*, *Ps. pyocyanea* are the best-known representatives. Their metabolism is a little different; amins and ammonia are formed, while hydrogen sulphide, phenol and indol are absent. As a third group, *B. coli* may be mentioned which forms indol, but no ammonia from peptone, and whose proteolytic powers are very weak as it does not even liquefy gelatin.

Anaerobic decomposition of proteins is limited to very few species; there is a great difference in the availability of proteins and of carbohydrates as a source of energy, protein being available only to a few species, most of these preferring carbohydrates if they are present together with protein. *B. putrificus* is the main representative, but other forms exist. *B. putrificus* is strictly anaerobic, and a spore former, very common in nature. Among the products are skatol, hydrogen sulphide, ammonia and other very offensive compounds.

UREA, URIC ACID, HIPPURIC ACID, are the end products of protein metabolism of the higher animals. The decomposition of urea to ammonium carbonate has been mentioned in several places, mainly on page 202. It is a simple hydrolysis

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

This change can be brought about by only a few bacteria which are commonly grouped together as "urea bacteria." These organisms have hardly anything else in common, however, and the group is not a well-defined one. There are rods and coccus forms, motile and non-motile organisms, spore-formers and non-spore formers, and even molds have recently been found to hydrolyze urea. All urea bacteria can live without urea, feeding on organic matter like other bacteria, but most of them require an alkaline medium.

Hippuric acid is split by certain bacteria to benzoic acid and amino-acetic acid which can be oxidized completely. Uric acid can be changed in several ways. In some of these changes, urea is found as an intermediary product.

PRODUCTS FROM MINERAL COMPOUNDS

Minerals are used freely by microörganisms for cell construction, consequently, they do not leave the living cell like fermentation products. But a few organisms can actually decompose mineral matter and when this takes place mineral products are secreted. Two main processes can be distinguished, oxidation and reduction.

OXIDATIONS are the result of the organisms seeking a supply of energy. Several oxidations of minerals have been indicated previously, as the oxidation of ammonia to nitrites, of nitrites to nitrates, of hyposulphites to sulphates, of hydrogen sulphide to sulphur and of sulphur to sulphuric acid, of ferrous salts to ferric salts. All these microbial changes are simple processes and can be followed by chemical analysis much more easily than organic fermentations. The organisms which cause these changes, do not, as a rule, thrive in organic substances and for this reason pure cultures can be obtained only with difficulty. Their activity is of great importance in soil fertility.

REDUCTIONS of minerals, too, are of great significance. As a typical example, nitrates may be reduced to nitrites, to ammonia, to nitrogen gas, and, rarely, to nitrogen oxides. The reduction may be performed either by the direct removal of oxygen, or by the formation of free oxygen. The reduction of nitrates to nitrites can be written in the following three ways:

$$\begin{aligned} KNO_3 - O &= KNO_2 \\ KNO_3 &= KNO_2 + O \\ KNO_3 + 2H &= KNO_2 + H_2O. \end{aligned}$$

The result in all three cases is the same. Many bacteria can reduce nitrates to nitrites or to ammonia. A few can reduce them to nitrogen.

These "true denitrifiers" are found in soil and in old manure. Their reducing process is as follows:

$$Ca(NO_3)_2 - 5O = CaO + 2N.$$

Nitrates are reduced through the efforts of the organism to secure a supply of oxygen. The denitrifying bacteria have strong oxidizing properties; they take oxygen from all sources possible. If cultures of denitrifying bacteria are well aerated, as in soils with a proper moisture content, they scarcely attack the nitrates, while they will reduce them in ordinary liquid cultures so fast that the escaping nitrogen gas forms a froth on top of the nitrate solution. Denitrifying bacteria need the oxygen to oxidize organic matter. They cannot live without organic food.

Sulphates are reduced in a very similar way to hydrogen sulphide

$$H_2SO_4 - 4O = H_2S.$$

Tap-water, containing calcium sulphates, often forms hydrogen sulphide if shut off from the air for some time.

While only a few bacteria reduce sulphates, many reduce sulphites or sulphur to hydrogen sulphide. The potassium and sodium salts of selenic and telluric acid (H_2SeO_4 and H_2TeO_4) are reduced by certain organisms and not by others. The reduction results in a colored precipitate; this reaction has been suggested as a diagnostic means to distinguish different species. The reduction of arsenious oxide to arsin (AsH_3) is used as a very delicate test for arsenic; it is applied in the detection of arsenical poisoning. The material to be tested is sterilized and inoculated with *Penicillium brevicaule* (page 53, the "arsenic mold"). This will reduce most arsenious compounds to arsin (AsH_3) or to diethyl arsin, $AsH(C_2H_5)_2$, both of which are easily recognized by their very pronounced garlic odor.

Unknown Products of Physiological Significance

Among the products of microbial action, there are certain substances which must be mentioned because of their importance, though their quantity is insignificant compared with the ordinary products of fermentation. These substances can be divided into four groups: *pigments*, *aromatic compounds*, *enzymes*, and *toxins*. The chemical structure of pigments and of many aromatic substances is scarcely known; and as far as enzymes and toxins are concerned, it is not even determined whether or not they are of protein nature. The last two groups are known only by their actions, while the pigments are very conspicuous and cannot possibly be overlooked.

PIGMENTS have naturally attracted the attention of microbiologists ever since pure cultures were known, and many investigators have tried to explain the nature and the meaning of pigments. All experiments concerning the purpose of pigment-formation by microörganisms have been without results. It is not known that the pigment is of any material advantage to bacteria; for it is possible to cultivate colorless strains of pigment bacteria which grow apparently as well as the original pigmented culture. Again, pigments cannot take the place of the chlorophyl in plants except perhaps the bacteriopurpurin of the purple bacteria. It does not even protect the cells against intense light, because the pigmented organisms are not more resistant than the corresponding colorless "sports." The only exception are the colored spores of the molds, especially *Penicillium* and *Aspergillus*, which are very resistant to light, while the spores of *Oidium* are killed just as easily as the mycelium. Pigments cannot be considered as reserve substances, since many pigments are excreted and remain outside the colorless cells. Pigment production may be incidental. It is possible that the waste products of certain organisms happen to be colored.

After Beyerinck, the chromogenic bacteria may be divided into three classes:

1. *Chromophorous bacteria*, in which the pigment is placed in the cell and has a certain biological significance analogous to the chlorophyl of higher plants. In this division belong the green bacteria discovered by Van Tieghem and Engelmann and the red sulphur bacteria or purple bacteria.

2. *Chromoparous or true pigment-forming bacteria*, which set free the pigment as a useless excretion, either as a color-body or as a leuco-body which becomes colored through the action of atmospheric oxygen. The individuals themselves are colorless and may under certain conditions cease to form pigments. To this class belong *B. prodigiosus*, *B. cyanogenes*, *Ps. pyocyanea*, and others.

3. *Parachrome bacteria*, which form the pigment as an excretory product but retain it within their bodies, as *B. janthinus* and *B. violaceus*.

When the pigment is soluble in water, as those produced by *Ps.*

pyocyanea and the fluorescent bacteria, it diffuses through the medium. When the pigment is not soluble, it either lies within the cell wall or between the individuals.

This classification furnishes some details concerning the methods of pigment production, which depends upon the presence of certain media. According to Sullivan, sometimes certain mineral salts, sometimes sugar will stimulate chromogenesis. The same is true with molds. Very brilliant colors appear with certain species of molds if grown on cellulose or on fat, while on gelatin the pigment is not produced. The temperature is an important factor. A large number of chromogens produce no pigment when grown in the incubator. It is possible to obtain non-pigmentation with many species by propagating them through many generations at high temperatures. Oxygen also is necessary for the chromogenesis of many bacteria. Some need a short exposure to daylight in order to produce their pigment, while cultures grown in absolute darkness may remain colorless. Strong sunlight, however, will check pigment production in the same degree as do antiseptics and other harmful influences.

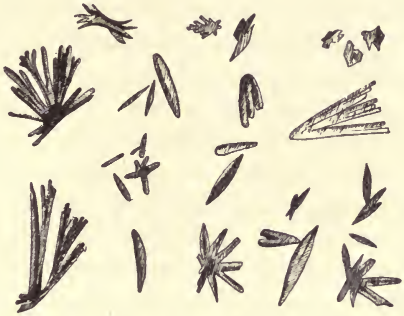


FIG. 113.—Bacteriopurpin, from a *Rhodospirillum*, crystallized from a chloroform solution. (*After Molisch.*)

The chemical nature of microbial pigments is little known. They are distinguished according to the solubility in various liquids, as water, alcohol, ether, chloroform, benzol, and other solvents, and according to the change of color caused by acid and alkali. A group of *carotin bodies*, named because of their similarity to the pigment of carrots, the *prodigiosin bodies*, named after *B. prodigiosus*, the fluorescent pigments and perhaps a few other groups are distinguished, but their chemical nature is rather vague as yet. The absorption of distinct lines of the spectrum by solutions of these pigments is claimed to be a very reliable means of distinguishing the pigments of different species.

AROMATIC SUBSTANCES constitute another group of metabolic products. The chemical analysis accomplishes more with these compounds than with pigments, since they are frequently well-known compounds. The main difficulty arising in their identification is in the very minute quantities of the products available. Some substances with strong, mostly very disagreeable odors have already been mentioned: indol, skatol, hydrogen sulphide, mercaptan, the amins and ammonia, butyric acid, and some of the higher alcohols. There remain to be mentioned certain oils and esters giving rise largely to pleasant aromas. The formation of aromatic oils has been established although their nature is entirely unknown. The same is true with the esters. The substance causing the fishy flavor in butter is volatile with steam and is neither of an alkaline nor acid nature. The strong odor of freshly plowed earth is caused by an *Actinomyces;* the odor can be traced to a very volatile oil the nature of which has not been determined. The aroma of fermented liquids—wines, beers, and many others—is partly due to compounds constituting the fermenting material, and partly to the fermenting agent. Some yeasts are known to produce fruit-esters, as succinic-acid-ethylester and the corresponding esters of malic and other acids. Besides, some glucosides may be split and traces of hydrocyanic acid and benzoic acid may be liberated. The change of flavor with the aging of wines is probably more a chemical than a biochemical change.

ENZYMES AND TOXINS.—Among the most interesting and least understood products of microbial action are the *enzymes* and the *toxins.* These two groups are related in many respects The enzymes have been discussed extensively in a preceding chapter and toxins will be treated more extensively on pages 676, 740. Toxins and enzymes are formed by the cells in such small quantities that they would never have been discovered by ordinary chemical means were it not for the unusual effects which they produce, the enzymes acting upon food substances, and the toxins acting physiologically upon organisms. Toxins and enzymes are chemically unknown. It is assumed that they are chemical bodies, but even this has not been proved. A pure toxin has never been obtained and we have no criterion for its purity. The presence of a toxin is recognized only by an animal test and in this way the comparative concentration can be determined approximately. Such standardization of toxin solutions is only comparative, however, and gives no clue as to the actual amount of toxin present. Not all animals are sensitive to all toxins. It is quite possible that all bacteria produce compounds with chemical qualities similar to toxins, and only a few of them happen to react upon men or animals.

Toxins are not always the product of microbial action. Vegetable toxins or *phytotoxins* are known, among which the ricin of the castor-oil bean is perhaps the most studied representative. The best-known zoötoxin is the rattlesnake poison. These non-microbial compounds have the same quality as the microbial toxins—they are extremely poisonous. Toxins are the cause of disease in diphtheria, tetanus and botulism. If a culture of these organisms is filtered through a porcelain filter which removes all bacterial cells, the filtrate injected into an animal will cause the disease with all its accompanying symptoms though there are no microörganisms introduced into the animal body. If the filtrate is heated, however, no effect will take place after the injection, because heat destroys the toxin. The amount of toxin that will kill an animal is extremely small. .000005 mg. of the purest tetanus toxin will kill a mouse, .0007 mg. of ricin will kill a rabbit, less than .23 mg. of tetanus toxin will kill an adult man. The body of an animal or man forms an anti-body against the toxin which neutralizes its poisonous action. Anti-bodies are also formed against enzymes injected into an animal.

Toxins are very sensitive to heat. A short exposure to temperatures between 80° and 100° will inactivate them. They are also very sensitive to light. While some toxins are secreted, others are retained within the cells of microörganisms, and never leave them until the cells die or disintegrate. Ptomains, which are also metabolic products of microorganisms and sometimes cause poisoning, differ from the toxins in their resistance to heat and light (page 241). Ptomains differ in no way essentially from ordinary organic compounds; the animal or human body produces no anti-ptomains to counteract their poisonous effects. There is no chemical relation whatever between toxins and ptomains, and the physiological effects are also quite different, though they both cause poisoning.

Toxins are not essential products of the metabolism of pathogens. Strains of pathogenic bacteria can be bred which do not produce toxins as chromogens can be bred without pigment, or lactic bacteria which do not produce acid. The strains which lose their pathogenicity grow better on artificial media but are less able to produce disease in the animal. They may regain the power of producing toxin if passed through the body of the animal. The real object of toxin production by microörganisms is not known; the microörganisms derive no apparent benefit.

Physical Products of Metabolism

Production of Heat.—It has long been known that fermentation produces heat. The rise of temperature is usually not very great. In lactic fermentation it amounts to about 1°, in alcoholic fermentation to 2 or 3°, but in certain processes the heat liberated is considerable, as in the fermentation of manure, of ensilage, of vinegar, and in others.

The cause of heat formation is quite evident from the discussion on page 199. Decomposition of organic matter means a liberation of energy which is used for the continuation of life processes; the utilization is, as a rule, incomplete, and a part of the energy appears in the form of heat. The amount of heat produced can be measured directly with the thermometer if great care is taken that no heat is lost by radiation or by evaporation of water.

Much heat is produced in the vinegar fermentation. In the quick-vinegar process (page 644) the temperature rises sometimes as high as 10° to 15° above the temperature of the room and the vinegar manufacturer uses the heat produced by the bacteria to keep the generators at the optimum temperature. If the process is not controlled carefully, the vinegar bacteria are likely to produce sufficient heat to kill themselves.

The heat produced in the fermentation of manure, especially horse manure, is used in the hot-beds to cultivate and force young plants. In the manure pile, great heat production is not desirable because high temperatures will volatilize the ammonia; the tight packing of manure which keeps out the oxygen will prevent too strong bacterial action. The highest temperature in silos which has been recorded is about 70°, but the best silage is secured by keeping the temperature below 50°. Ensilage fermentation is not thoroughly understood, however, and no accurate statements can be made as to the cause of the increase in temperature. Sometimes the temperature in silos does not exceed 35°. The curing of hay is usually accompanied by a rise of temperature. For some time it was believed that the spontaneous combustion of hay was mainly due to microörganisms, but it has been shown recently that even sterile hay will show a rise of temperature under certain conditions. This does not exclude the formation of heat in hay by microörganisms under other circumstances. The heating of tobacco, of green or moist grain or corn is not of bacterial origin, but due to the respiration of the living plant-tissue.

Production of Light.—The light-producing or photogenic organisms are quite numerous and occur more frequently than is generally believed. The phosphorescence of decaying tree stumps and leaves in the woods and of meat and fish in the cellar are well-known phenomena. The phosphorescence of wood and leaves is generally caused by *Hyphomycetes;* certain mushrooms have this quality in a very high degree. The light of meat and fish is usually generated by bacteria, of which at least twenty-six species have been described.

"The most obvious evidence of liberation of energy in the physiology of protozoa is seen in their movement. Certain protozoa, *Noctiluca* for example, however, emit light and produce the phosphorescence often observed in sea water. From analogy with higher animals it is to be supposed that heat and electrical changes are also produced."

Many experiments have been carried on in order to discover the nature and origin of the light, but, so far, few results have been obtained. The phosphorescence is due to an oxidation process; all photogenic organisms cease to generate light when the oxygen is removed. As soon as they come into contact with oxygen again, they produce light immediately, and this sudden flashing is used occasionally by physiologists as a very delicate test for oxygen. The light appears to be produced always within the cell; no cell product has ever been found to give rise to light outside the cell. It is possible that a chemical compound is formed in the cell which generates light when in contact with oxygen.

The life processes of the photogenic microörganisms are not necessarily connected with the formation of light. Photogenic bacteria are known to lose the power of light production as the chromogenic bacteria may lose the power of pigment production. Phosphorescence has, like pigmentation also, no bearing upon the development of the cell, and the light-giving compounds may be regarded as incidental waste products. Certain chemical bodies stimulate light production, while others favor the growth only. One of the most important factors in the production of light is sodium chloride.

CHAPTER V

PHYSIOLOGICAL VARIATIONS ASSOCIATED WITH METABOLISM AND NUTRITION*

The great variability of microörganisms in morphological respects has already been pointed out in Part I of this book. A similar variation and adaptation are noticed in their physiology, especially with the food substances of bacteria and consequently with their metabolic products. Microörganisms change their physiological properties very readily with the environment; the new variety may keep its acquired properties for some time even if brought back to the original conditions. It is stated frequently that microörganisms tend more toward variations than the more complex organisms. It should be considered, however, that the experiences in the variations of green plants and animals are based on individuals, while in the case of microörganisms these experiences are gained almost always from millions of cells. A simple illustration is the development of bacteria in salt solutions. If a broth culture of *B. coli* is transferred into broth containing 8 per cent of salt, a large number of cells will die, often more than 99 per cent. The surviving bacteria begin to multiply after a certain length of time and a new variety is created which can tolerate the salt. At first, only about one out of one hundred cells had the power to tolerate salt, but, since the dying cells are not usually counted or considered at all, it is customary to say that bacteria easily adapt themselves to an 8 per cent salt solution. If only one single plant out of one hundred could be adapted to a certain high temperature, it could not be said that it adapts itself easily. This mistake is quite commonly made with microörganisms.

The best illustration for the variability of cultivated microörganisms is the enormous number of varieties of *Saccharomyces cerevisiæ*. Nearly every large brewery has a yeast type of its own which differs from others by the amount of alcohol and aromatic substances produced, by time and optimum temperature of spore-production, by the appearance of the budding yeast in the hanging drop, and also in other respects. The cultivated organisms are not alone in showing this tendency toward variation. The transferring of a soil or water bacterium into the ordinary laboratory media is a complete change of conditions; the different cells of the same species may react differently and give several varieties. A lactic bacterium on meat medium without sugar does not thrive well in the first generations, but it gradually becomes able to grow on this medium. By this treatment, it loses gradually the power of producing acid and does not thrive as well in milk. The attenuation of pathogenic bacteria by cultivation on media, as potato, very different from the blood and muscle upon which they grow most naturally, or by growing them at low temperature, or above the maximum, furnishes another example. The decrease and finally the entire loss of pathogenicity is caused by a change of metabolism, by a loss of the power to produce toxin.

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* Prepared by Otto Rahn.

As by certain diet the metabolism can be changed, so certain physiological properties of bacteria can, by proper cultivation, be increased. By the frequent transferring of an organism on gelatin, its liquefying qualities can be increased, provided it had some at the start. By continued passing of a bacterium through an animal, its virulence can be increased. Strains of bacteria which will produce a very high acidity can be bred; this is illustrated by the quick-vinegar process and by the strong alcohol-producing yeasts of the distillery process. By continued cultivation of an organism upon a certain medium, it will become so acclimatized that it degenerates readily when the conditions become unfavorable Such specifically trained strains of microörganisms are used in alcoholic and lactic fermentation, in pathogenic bacteriology and in the inoculation of leguminous plants with nitrogen-fixing bacteria.

Factors Influencing the Type of Decomposition

In the chapter on products of metabolism, it has been shown that the same compound can be decomposed in many different ways, and the question may well be asked what decides the type of decomposition. Since bacteria are widely distributed, it must be expected that there are certain conditions which are most favorable to a given type of fermentation, while under changed conditions, other types are more likely to dominate. The fact that sugar in cider nearly always undergoes alcoholic fermentation, while in milk it undergoes lactic fermentation, has its reason in the physiology of the bacteria, and in their reaction upon the environment.

Cider is acid, and acid is not well suited for the growth of most bacteria. The vinegar bacteria can grow in fruit juices, and a few other bacteria, especially those causing trouble in wine, are not retarded by fruit acids, but the common types attacking proteins and causing organic decay are not able to grow on fruits. Yeasts, however, and molds thrive well only in acid media. They can exist in neutral solutions if in pure culture, but in nature they are easily crowded out by bacteria. Acidity of the medium is therefore one of the most important factors regulating the type of microbial decomposition. This principle is commonly utilized by preserving foods of all kinds in vinegar, and by making butter from sour cream rather than sweet cream; the keeping qualities of hard cheeses depend upon their acid content.

In acid environment, the two most common types of decomposition are oxidation, complete or incomplete, and alcoholic fermentation. The oxidation is brought about by molds or organisms closely allied to yeasts. The latter are very common on all sour foods, especially on foods containing lactic acid, such as cottage cheese or sauerkraut. The kind of acid decides the type of mold; wherever there is lactic acid, there is *Oidium*, while malic and tartaric acids favor *Penicillium* and *Aspergillus*.

If the decaying materials contain no acid, the type of decomposition depends mainly on the presence or absence of carbohydrates, especially sugar. It is an old experience, recently verified through a large number of experiments by Kendall and Walker, that practically all bacteria will decompose sugar in preference to proteins. If a leaf contains sugar and protein (cabbage) the sugar decomposition will be conspicuous, and the protein is not attacked very readily. Putrefaction in the presence of sugar or of acid does not take place. Meat will not putrefy if mixed with sugar, while milk putrefies readily if the sugar is removed by dialysis. The three types of sugar decomposition which come into consideration in neutral media, are the lactic, the acid-gas and the butyric fermentations. The latter is a strictly anaerobic fermentation, and thus limited to special conditions. Of the other two, the acid-gas fermentation is the most common, and the souring of vegetables of all kinds is due to this type of fermentation (pickles, sauerkraut, ensilage, salt-rising bread). Sometimes the acid-gas fermentation is followed by a butyric fermentation. The true lactic fermentation is not common, and is limited almost entirely to milk. This is explained by the circumstance that the organisms causing this decomposition are parasitic in their habits, causing disease or living in the intestine of animals. In the absence of acid and sugars, putrefaction is the most common type of decomposition.

Many factors aside from the chemical composition of the medium are essential. Oxygen has already been mentioned as preventing butyric fermentation. It will also prevent the acid-gas fermentation if too abundant. Ensilage is trampled and pressed down to avoid air spaces as much as possible, for molds will outgrow the acid-forming bacteria if air has free access. Absence of oxygen will prevent mold growth, and for this reason, jelly is paraffined, and butter wrapped tightly into impermeable paper. The influence of oxygen upon the type of protein and of cellulose decomposition has been pointed out previously.

The moisture content is of great importance. As will be shown later, not all organisms have an equal need of moisture; some molds will grow on foods too dry for bacteria and yeasts. Molds are especially adapted for growing on dry media, as only part of their cell substance is immersed in the medium. Their thread formation enables them to search a dry medium, such as flour, for moisture, the extreme of adaptation being *Rhizopus*, and the construction of the fruiting bodies shows that they are destined by nature to be spread by air and wind. It is no wonder that damp organic matter, if it can be decomposed at all, will show molds, and nothing else, regardless of the chemical composition, for there is no competition. Flour, moist seeds, incompletely dried fruit, damp milk powder will always become moldy. The same holds true with very concentrated sugar solutions such as syrups, jellies and jams, while in concentrated salt solutions, molds cannot thrive, and the torula yeasts are best adapted to such conditions.

A very important part is also the structure of the material. Microorganisms act mainly upon organic matter, and since this comes from living organisms, it has usually definite structure, exceptions being milk and blood. The structure of all living organisms is such as to prevent the intruding of microörganisms. The body of plants and animals is surrounded on the outside by tough and dry layers of epithelial cells, and the cavities of the animal body also have their protective membranes. Microörganisms cannot enter the tissues if these membranes are perfectly sound, and we know that, as a rule, the tissues of healthy plants or animals are free from bacteria. Thus, a healthy apple or potato or egg will not be infected and decomposed by microörganisms if handled carefully, meat will begin to decompose on the outside, and the inner parts may be still good when the outer layer is already in a state of decay.

In the plants, each cell is surrounded by its special cell membranes which are a barrier to infecting organisms. If we prick the skin of a healthy apple with a pin infected with yeast, the infection will not spread though we know that yeast will grow most abundantly in cider; in the apple, however, it has no means of spreading from one cell to the other. Molds possess this means; they can puncture cell walls, and forcing their way from one cell to the other, they will soon bring about the rotting of the entire fruit after it once becomes infected. This protection seems especially necessary in the plant's roots which are greatly exposed to injury from insects and other animals in the soil and surrounded by billions of microörganisms. They are attacked only by fungi which can force their way from cell to cell, or by bacteria which can dissolve the membranes by means of enzymes, and thus cause a softening of the root tissue. The bacteria causing the various rots of vegetables belong to this type.

There is, then, a great variety of factors deciding the type of decomposition of organic matter in nature, and by knowing the chemical composition as well as the structure and other physical conditions, it is possible to foretell which group of organisms is most likely to attack the compounds in question.

Another quite important factor, the temperature, will be discussed in more detail in one of the following chapters.

CHAPTER VI

NUTRITION OF MICROORGANISMS AND THE ROTATION OF ELEMENTS IN NATURE*

All organic matter on earth is undergoing continuous change. Organisms grow and decay. The same carbon and nitrogen atoms which constitute the organic world of to-day constituted it thousands of years ago. The amount of carbon, nitrogen, hydrogen and of all other elements of life on earth is limited, and the same atoms will be used for the future generations of life that constitute the present. There must be continuous destruction to enable new construction. Construction is mainly the task of green plants, enabled by the chlorophyl to use the energy of sunlight in building up organic substances from minerals, water and carbon dioxide. Destruction is caused mainly by animals and other organisms which have to break down organic matter in order to exist. These two factors keep the atoms of the organic world in perpetual rotation.

In this circulation of the elements it is necessary that all compounds of organic nature be decomposed finally to a form available for plant food. If this were not the case, the indestructible compound would sooner or later accumulate in such enormous quantities that the elements constituting this body would be removed entirely from general circulation. Let us suppose, as an illustration, that for some unknown reason, all urea bacteria on earth would die. Urea could be decomposed no more, and the plants, unable to use urea as a source of nitrogen in place of nitrates, would get but little benefit out of stable manure. All urea would pass gradually undecomposed into rivers, lakes, and finally into the ocean where it would accumulate continuously. The enormous quantities of nitrogen taken out of circulation would cause a decreasing growth of plants, and life would soon cease because of lack of nitrogen. For this reason all products of living organisms must be further broken up by some other organisms, and we find that the destructive work is to a large extent the task of microörganisms. Many products of organic life cannot be broken down by organisms other than bacteria, and therefore bacteria are absolutely necessary for the circulation of the elements and for life on earth. Bacteria and green plants are an absolute necessity for the maintenance of life, the one breaking down, the other building up, one dependent upon the products of the other; animals, however, could be excluded from the circle without interfering with a continuation of life on earth.

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* Prepared by Otto Rahn.

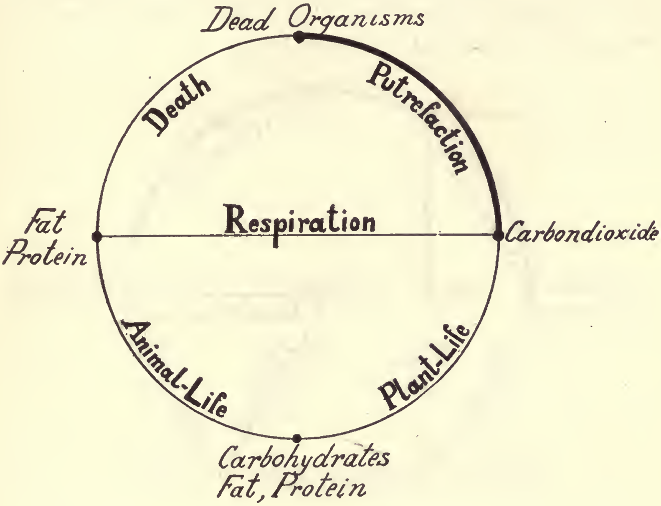

FIG. 114.—Carbon cycle.

CARBON CYCLE.—Carbon is the main element in organic nature, and the study of its cycle might be begun with its simplest compound, the carbon dioxide of the air. It is absorbed in this condition by the green plants, and is changed by the chlorophyl granules of the leaves to organic compounds of various types, either to carbohydrates (cellulose, starch, sugars) or to fats, or to protein substances, occasionally to organic acids or other compounds. The plants will either die and decay, or will be eaten by animals. In the first case, the decay will be caused exclusively by microörganisms; if the plants are eaten, they will be digested; part may be used to build up the animal body or stored as reserve substances, largely fat and protein. If the animal dies, a decomposition process will take place, which breaks down the organic compounds to simpler products and finally the carbon will be completely oxidized to carbon dioxide. Even the marsh gas which might be liberated in this process will find organisms that oxidize it to carbon dioxide and water. Every product will find an organism to break it up further until it is completely disorganized and the carbon atoms can start the same circulation anew. Undoubtedly as long as organic life has existed on earth, microörganisms have been present, in order to render the dead organic matter again available for plant and animal life. Fig. 114 gives a schematic illustration of the carbon cycle; the microbial activity is marked by heavy lines.

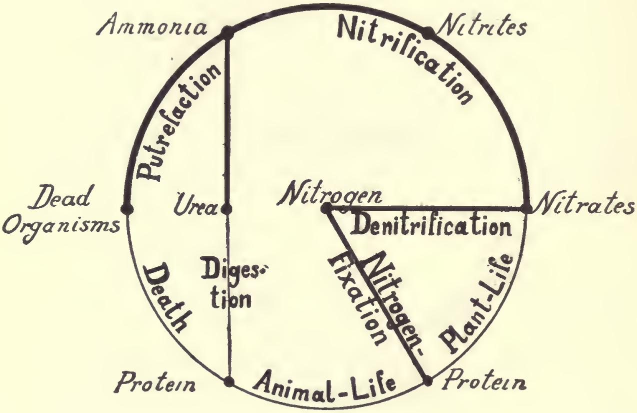
Ammonia
Nitrification
Nitrites
Putrefaction
Dead Organisms
Urea
Nitrogen
Nitrates
Denitrification
Digestion
Death
Nitrogen-Fixation
Plant-Life
Protein
Animal-Life
Protein

FIG. 115.—Nitrogen cycle.

NITROGEN CYCLE.—Nitrogen shows the same continuous change as carbon. Plants take up nitrogen in mineral form usually as nitrates. The plants change this mineral nitrogen to the most complex bodies, proteins, where it is combined with the other elements of organic nature. The plants may be eaten by animals; part of the protein is then digested to urea or hippuric or uric acid, which in turn are readily decomposed by microörganisms to ammonia (Fig. 115). Part of the protein will be stored in the growing animals, and if the animal dies, the body will decay or putrefy, and the nitrogenous compounds of that body will pass through the various stages of decomposition to the final product, ammonia. Ammonia is then oxidized to nitrites and nitrates, when the nitrogen cycle is completed.

There is, however, one discrepancy in this cycle. It has been mentioned already that some organisms are able to reduce nitrates to nitrogen gas. This is one of the "leaks" in the rotation of elements which would be disastrous to organic life on earth if there were no means to compensate for the loss of nitrogen in circulation. Imagine what would happen if there were no such compensation. Part of the nitrate in the soil is destroyed, the nitrogen gas escapes into the air and is as indifferent as the nitrogen of the atmosphere lost to organic life forever.

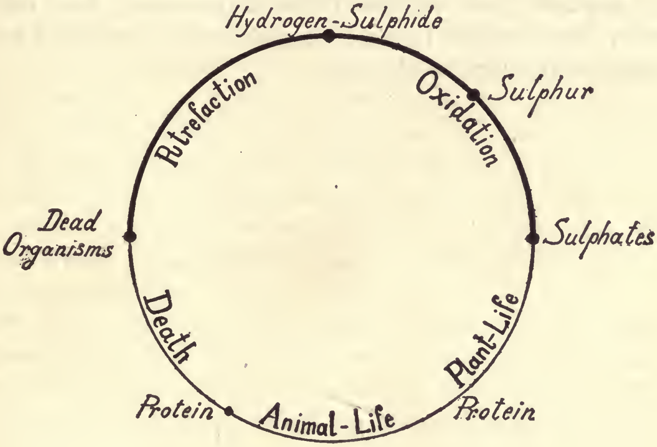

FIG. 116.—Sulphur cycle.

More nitrates would be produced from decaying organic matter and would eventually be destroyed. After a certain time, this continuous loss of nitrogen would become quite noticeable in the growth of plants; there would be a scarcity of nitrogen in soil, since part of it is lost continuously. Finally, the plants would cease to grow because the nitrogen in the soil would be exhausted.

The compensation for this destruction of available nitrogen is found in the nitrogen-fixing bacteria, which, either living in symbiosis with leguminous plants or growing independently in the soil, have the power to use the atmospheric nitrogen for the formation of their own protoplasm. Thus, organic nitrogen is produced from nitrogen gas and the continuance of organic life is guaranteed.

Sulphur Cycle.—Little more can be said about sulphur, since the rotation is quite similar to that of nitrogen. Plants will take sulphur usually in the form of sulphates and make protein compounds containing a certain amount of sulphur (Fig. 116). These bodies are either digested by higher animals or broken down by putrefaction to the final product, hydrogen sulphide, which is oxidized by the sulphur bacteria first to sulphur, then later to sulphates.

Phosphorus Cycle.—The cycle of phosphorus has not been worked out completely, but from the discussion in the last pages, it is plainly seen that a simple cycle very much like the ones above must exist. It is probably much simpler because phosphorus does not enter as easily into organic compounds as nitrogen.

DIVISION III

PHYSICAL INFLUENCES

CHAPTER I

WATER AS A PHYSICAL FACTOR*

It has been indicated already that water has the capacity as a solvent way beyond any other substance; it has a function, closely associated with its solvent powers as a carrier, in which solution and mechanical mixture are equally important; it possesses the property of diffusion, which enables its solutions to extend where other solvents find no entrance; it possesses much surface energy, having a very high rating; and it fosters ionization, the full value of which in life's reactions is not known. Living cells have been shown to consist of a high percentage of water. It appears as if water were the body-medium for all physiological reactions. [See pp. 187]

OSMOTIC PRESSURE.—In the organic world we find very commonly membranes which will allow water to pass through but retain some compounds dissolved in the water. Such so-called semi-permeable membranes are found surrounding the protoplasm of cells. They are not the cell wall, but separate the protoplasm from the cell wall. Similar properties are found in parchment paper, pig's bladder, and other organic membranes.

If a salt solution is poured in water, the two liquids will mix in a short time and soon every smallest portion of the mixture will have the same concentration. If a salt solution and water are separated by a membrane which does not allow the salt to pass, the water will go through the membrane toward the salt with a certain amount of pressure. This pressure depends upon the nature of the dissolved substance as well as upon its concentration.

The pressure increases in direct ratio with the number of molecules in solution. Therefore, a compound with large molecules (cane sugar) will produce a lower osmotic pressure than one with small molecular weight (glycerin) if we compare solutions of equal concentration. The osmotic pressure of protein, starch and peptone solutions can be measured only with the finest instruments, while the pressure of a 30 per cent dextrose solution is 22 atmospheres.* [See pp. 173-177.]

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* Prepared by Otto Rahn.

Plasmolysis.—If a cell is brought into a strong solution of a substance which cannot pass the plasma-membrane, this substance will cause an osmotic pressure and the concentration in the cell being lower than in the medium, the water will pass out from the cell until the pressure inside and outside is the same. This causes a shrinking of the protoplasm, while the rigid cell wall keeps its shape. Such plasmolyzed organisms are illustrated in Fig. 69, page 89.

While plasmolysis is easily demonstrated with the cells of higher plants, microörganisms do not show it so readily. In fact, many bacteria, like *B. subtilis*, *Bact. anthracis*, cannot be plasmolyzed by any concentration of salt in solution. Others, as *B. coli*, *B. fluorescens*, react promptly. But even though many are killed, the rest recover from plasmolysis after a few hours, and appear normal. This indicates that the salt passes slowly through the plasma-membrane and thus increases the pressure inside the cell until finally the inside and outside pressure are the same again.

The fact that many microörganisms show no plasmolysis whatever is explained in the same way. These organisms probably have plasma-membranes so constructed that the salts diffuse through nearly as fast as the water. An absolute exclusion of all soluble substances by the membrane is impossible since the food can get into the cell only by diffusion through the membrane.

The resistance of various microörganisms against concentrated solutions depends upon the organism as well as upon the dissolved substance. The sodium and potassium salts of the common mineral acids act upon a culture nearly in proportion to their osmotic pressure, but the potassium salts always retard growth a little less than the sodium salts. The effect of salts upon microörganisms is therefore not due to the osmotic pressure only; the chemical constitution of the salts also plays an important rôle.

The different functions of life are influenced in different degrees by concentrated solutions. Some bacteria will multiply but not form spores in salt solutions. Molds will sometimes show a good growth in concentrated sugar solutions but fail to produce spores. *Bact. anthracis* loses its virulence in sea water. Often, the form of microörganisms is affected by concentrated solutions. Some bacteria grow more spherical, others become elongated or distorted. The deforming influence is not due to the osmotic pressure only, but depends mainly upon the chemical character of the salt; magnesium salts especially have a tendency to produce such involution forms.

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* One atmosphere equals the pressure of 1 kg. per square centimeter or about 15 pounds per square inch.

Salt and Sugar Solutions.—Most experiments on the influence of concentrated solutions have been carried on with sodium chloride, because of its wide application in the preservation of foods. Most microorganisms, especially the rod-shaped bacteria, are suppressed by a salt concentration of 8 to 10 per cent. At 15 per cent only few cocci develop slowly, while some species of *Torulæ* grow without a very noticeable retardation. Above 20 per cent the *Torulæ* are practically the only organisms which can develop. They are, therefore, found in all food products which are preserved by salt, as salted pork, beef, fish, butter, and pickles, often in nearly a pure culture. It seems that they are easily overpowered by other organisms in the absence of salt, but in salted food, this competition is eliminated.

The selective influence of salt is used in some fermented products to prevent undesirable fermentations. This is true in sauerkraut and brine pickles, where the desirable bacteria can grow in the presence of salt while the undesirable ones are kept away. Possibly the salting of butter has the same effects.

Another compound of great practical importance is cane sugar, which is the standard preservative for fruits and condensed milk. Its action has been studied mainly upon molds. Theoretically, dextrose should be expected to have twice as strong a preserving action as saccharose because it has only half the molecular weight and consequently produces twice as strong an osmotic pressure in the same percentage of concentration. Its preserving effect is indeed a little higher than that of saccharose, but the proportion is not nearly 1:2. The common molds are extremely resistant to strong sugar solutions, about 60 to 70 per cent of cane sugar seems to be the limit of growth for *Penicillium* and *Aspergillus* species. Yeasts can also grow and ferment in very concentrated solutions while bacteria in general do not tolerate solutions higher than 15 to 40 per cent, though many exceptions are known.

Colloidal Solutions.—In order to determine the amount of water which is absolutely necessary for microbial proliferation, only such media can be used which do not cause osmotic pressure. If *B. prodigiosus* does not develop in a 10 per cent salt solution, this is not due to lack of moisture, because the same bacillus will grow in a 30 per cent sugar solution which contains 20 per cent less moisture. Another factor besides the water content enters, which can be avoided only in solutions without osmotic pressure.

A few substances are known to give such solutions, namely, colloidal bodies which have a very large molecular weight. Their osmotic pressure even in very concentrated solutions would not be high enough to interfere with microbial growth. Among these colloidal bodies are found egg albumin, gelatin, peptones, all protein substances; also starch, dextrin and gum arabic among the carbohydrates. None of these substances has a retarding influence upon bacteria; some of them can be mixed with water in all proportions; consequently, they are the ideal medium to test the water requirements of microörganisms.

Experiments carried on with gelatin, powdered meat, crackers, bread and potato, vary but little in results. A few bacteria cannot grow in a medium with only 60 per cent water, but most organisms develop slowly even with 50 per cent water and some may be able to develop with only 40 per cent. Molds can grow very scantily in even more concentrated media. Protozoa probably have to have a more diluted medium for their development though no experiments bearing upon their water requirements are known to the author.

The fact that in a colloidal solution growth will cease if the moisture is below 30 to 40 per cent does not necessarily indicate the conclusion that any substance with less than 30 per cent water cannot be decomposed. The above statement refers only to solutions, while in natural media as dried foods or soil, a combination of solid and dissolved substances is involved. Butter is an excellent medium for many bacteria, yeasts, and molds, though it contains only 12 to 15 per cent of moisture. If butter fat were soluble in water, the concentration of 85 parts of solid in 15 parts of liquid would certainly prevent any growth whatever, but fat is insoluble, and the fat particles do not interfere at all with the growth of microörganisms in the droplets of buttermilk distributed all through the butter. The concentration in these small droplets is the deciding factor. If the growth of microörganisms in butter is to be prevented by salt, it is unnecessary to give any attention to the fat; the bacteria live only in the water and not in the fat globules. In adding 3 per cent of salt to a butter with 15 per cent of moisture, a brine of 3 parts of salt in 15 parts of water is produced; in other words, a 20 per cent brine, because salt does not dissolve in the fat. Similar considerations will come up in the preservation of fruit, vegetables, meat, milk, and other food substances by drying or condensation.

Desiccation.—Microörganisms do not die immediately after the removal of the water, and they do not die all at once after a given time. Death through drying is a slow and regular process. Paul and his associates found that the number of bacteria dying in the unit of time is, under constant conditions, proportional to the number surviving. If we had 1,000,000 cells per gram in the beginning, and the death rate were 90 per cent per day, there would be, at the end of each day, 10 per cent of the original number surviving. This would give the following numbers for one week:

Beginning	1,000,000 cells per gram.
After 1 day	100,000 cells per gram.
After 2 days	10,000 cells per gram.
After 3 days	1,000 cells per gram.
After 4 days	100 cells per gram.
After 5 days	10 cells per gram.
After 6 days	1 cell per gram.
After 7 days	0.1 cell per gram.

This table shows graphically the mode of death of dried bacteria. The number of cells approaches zero without ever (at least theoretically) reaching it. From one cell per gram after six days we do not come to 0 on the seventh, but to one cell in 10 g. and on the eighth day one cell in 100 g. The total number dying in the first day is much larger than that dying on the sixth day, but the rate is constant, 90 per cent of the number surviving. This regularity has been found with bacteria dying from various causes, and it is commonly compared with the simplest chemical processes, the monomolecular reactions.

Paul and his associates found further, that the death through drying is caused by an oxidation process; in pure oxygen bacteria died much faster. The poisonous effect of oxygen upon moist bacteria has already been pointed out on page 228.

Most resistant to drying are the spores of bacteria; mold spores, too, show considerable resistance, while some bacteria, *e.g.*, *B. carotarum* and *Ps. radicicola*, are readily killed.

The resistance of microörganisms is influenced greatly by the medium on which they are placed for drying. Hansen found that yeast cells dried on cotton were still alive after two to three years, while if dried on platinum wire some died in five days and others lived as long as 100 days. Compressed beer-yeast mixed and dried with powdered charcoal kept as long as ten years; *Ps. radicicola* dried on a cover-glass or filter paper died within twenty-four hours; on seeds, this same organism was still alive after fourteen days and in the dried nodules of legumes a few cells were able to reproduce after more than two years. Soil containing an average number of 17,000,000 bacteria per gram was dried for two years; the total number of organisms averaged then 3,250,000, 20 per cent of the bacteria, therefore, could resist desiccation. Dried cultures of microörganisms are commonly sold for several purposes, as dairy-starters and the so-called "magic yeast" and "yeast foam" used for bread-making. Such cultures are dried on milk, sugar, starch, flour or similar porous and absorbing material. Starters are usually guaranteed only for a certain length of time, from one to twelve months. The advantage of the dry culture is its better keeping qualities. Liquid cultures produce substances harmful to themselves, and die rapidly after a short time, while the dry cultures show little change.

The resistance of pathogenic bacteria to desiccation is of considerable importance in the spreading of contagious diseases. Many pathogenic bacteria die after desiccation of a few hours to a few days, and spreading of such diseases by dust is highly improbable. Protozoa of soil decrease in number by drying, but all are not killed.

CHAPTER II

INFLUENCE OF TEMPERATURE*

Temperature, as well as moisture, is one of the most important factors of life. It is so important that the most highly developed animals protect themselves by a very complicated mechanism of regulation against changes of temperature; the life processes of such animals will take place at a temperature nearly constant from birth to death. This causes the metabolism of warm-blooded animals to be different from that of all other organisms. The metabolism of the warm-blooded animals takes place at a constant temperature. The required amount of food is constant except for the part that is used for heating the body; at lower temperatures, more heat-producing material is used and the result is that warm-blooded animals require more food at lower temperature. All other organisms, reptiles as well as bacteria, have the temperature of their environment and the decrease of temperature will decrease the intensity of metabolism as it retards any other chemical process. The lower the temperature, the less food is required by all lower organisms.

There are, of course, limits to the favorable influence of high temperatures. Growth and metabolism of microörganisms will increase with rising temperature to a certain point, called the *optimum temperature*, and beyond this point the rate of growth will fall off rapidly and soon cease entirely. The highest temperature at which growth can take place is called the *maximum temperature*. Correspondingly, the *minimum temperature* of an organism is the lowest point at which growth can take place.

THE OPTIMUM TEMPERATURE which allows the fastest growth will be quite different for different species. Groups of bacteria are known which develop only at very high temperatures and others for which room temperature is too high. The temperature requirement is largely dependent upon the natural habitat of the organisms. The bacteria of the polar sea and of a lagoon near the equator will very probably have different optimum temperatures because of the acclimatization and selection which has been taking place for centuries.

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* Prepared by Otto Rahn.

The great majority of bacteria and related organisms, in fact of all living organisms, except in a few instances, has its optimum temperature between 20° and 40°. The optimum temperature of an organism is generally somewhat higher than the average temperature of its natural habitat.

The following table shows the data obtained for a few microörganisms.

Species	Temperatures		
	Minimum	Optimum	Maximum
Penicillium glaucum	1.5°	25°–27°	31°–36°
Aspergillus niger	7°–10°	33°–37°	40°–43°
Saccharomyces cerevisiæ I	1°–3°	28°–30°	40°
Saccharomyces pasteurianus I	0.5°	25°–30°	34°
Bacterium phosphoreum	below 0°	16°–18°	28°
Bacillus subtilis	6°	30°	50°
Bacterium anthracis	10°	30°–37°	43°
Bacterium ludwigii	50°	55°–57°	80°

THE MINIMUM TEMPERATURE or the lowest limit of growth is usually farther from the optimum than the maximum temperature. It will vary with the organisms just as do the other cardinal points. But there is a natural limit drawn by the freezing-point of the nutrient liquid. Not all organisms can grow at such low temperatures, in fact the greater number does not develop below 6° to 10°. Those that can grow at the freezing-point will be inhibited by the solidification of the water in the nutrient medium, for if the water is frozen, food cannot diffuse into the cells and therefore, all life processes are checked. If freezing is prevented by adding salts or other soluble substances which lower the freezing-point, growth may continue even below 0°. Milk freezes at about − 0.5°. Bacteria are found to multiply in it as long as it is not entirely solid. A certain yeast multiplied slowly in salted butter kept at about − 6°.

The number of microörganisms that developed at the freezing-point was found to be:

In 1 c.c. of market milk,	up to	1,000 germs.
In 1 c.c. of sewage,	up to	2,000 germs.
In 1 g. of garden soil,	up to	14,000 germs.

THE MAXIMUM TEMPERATURE is usually about 10° to 15° higher than the optimum. The development of microörganisms above the optimum temperature is not quite normal; there is a great tendency toward involution forms. The mycelium of molds grown near the maximum temperature appears unhealthy and pathogenic bacteria lose part of their virulence. This loss of virulence is made use of in the preparation of attenuated cultures for vaccines.

The maximum temperature varies with different species of bacteria. Most bacteria do not grow above 45°, but with some of them the maximum temperature is considerably lower. *Bact. phosphoreum* dies if exposed for a few hours at 30°; others may require still lower temperatures. The average organisms found in water, soil, milk, and the body, which have their optimum near 30° to 38°, do not grow higher than about 45°. There are very noticeable exceptions to these, such as the physiological group known as thermophilic bacteria.

These extraordinary organisms have their maximum between 70° and 80°, a temperature which coagulates albumin. Corresponding to the high maximum the thermophiles have a very high optimum, and the minimum lies with most of these species above 30°. These organisms are found in soil, sewage, ensilage and occasionally in milk. They find the temperature suitable for their life only under extraordinary circumstances, as in fermenting manure piles, in silos, in self-heating hay and similar organic material that develops a high temperature by fermentation. Some hot springs have a very remarkable flora of thermophilic bacteria.

The range of temperature within which growth is possible, is very uniformly 35° to 45°; the starting points and end-points of this range vary greatly, while the total range is quite constant, except for some bacteria adapted to special conditions, such as some pathogenic bacteria. The temperature relations of bacteria can be shown graphically by using as ordinate the rate of growth, as abscissa the temperature.

BIOLOGICAL SIGNIFICANCE OF THE CARDINAL POINTS OF TEMPERATURE.—The importance of the temperature requirements of certain organisms to the rôle they play in nature can be illustrated by a few examples. Most molds cannot cause disease in man and warm-blooded animals because their maximum temperature is below the body temperature. Exceptions are some *Aspergilli* and *Mucorineæ*. Pathogenic microörganisms must have their optimum temperature coincide with that of their host.

Organic substances may undergo a different change at different temperatures. The biochemical changes in soil may not be the same in northern Canada and near the Gulf of Mexico. Even the warm and cold season of the same climate is apt to change not only the rate of decomposition but possibly the products. Perhaps the most striking example in this respect is the decomposition of ordinary market milk kept at different temperatures. Such milk contains a great variety of microörganisms; at various temperatures different types will predominate, while the remainder are retarded or inhibited by unfavorable temperature conditions and by the products of the dominant type of bacteria. If milk is kept at about the freezing-point, only a few organisms will develop slowly, but after a certain time their number will increase to many million cells per c.c. There is, however, no apparent change; no acid or deterioration can be discovered by the taste though chemical analysis proves the presence of hydrogen sulphide and ammonia. Between 15° and 25°, milk will sour in about thirty-six to forty-eight hours, giving a firm curd of an agreeable flavor without whey or gas; later *Oidium lactis* destroying the acid develops on the surface. Near body temperature the milk will lopper in twenty-four hours, the curd is usually contracted, a large quantity of whey is extruded, and much gas is produced by *Bact. aerogenes* and *B. coli*. The odor is disagreeable and later butyric acid is produced; eventually the lactic acid increases further by the action of *Bact. bulgaricum*. If kept above 50° the milk either keeps permanently, or a decomposition by thermophilic bacteria begins which is either an acid fermentation followed by digestion or a complete putrefaction, depending upon the species of thermophilic organism that happens to be in the milk sample. Thus there can be induced in the same substance, containing the same organisms at the start, four entirely different types of decomposition merely by the difference of temperature.

This indicates the importance of temperature regulation in the fermentation industries. Even pure cultures may give different products if working at different temperatures. Cream ripened with a pure culture starter at too high a temperature will have a sharp acid flavor. The cold curing of cheese has become a very common practice because of the much improved flavor. Bioletti claims that the value of the dry California wines would be doubled if the fermentation were carried on generally at a lower temperature.

End-point of Fermentation.—Another question is the relation between the end-point of fermentation and the temperature. Of the few data existing, many indicate that at a lower temperature the final fermentation goes farther than at a higher temperature. Müller-Thurgau found that under exactly the same conditions with the temperature as the only varying factor the following final amounts of alcohol were produced by a pure culture of yeast:

At 36°................	3.8 per cent alcohol.
At 27°................	7.5 per cent alcohol.
At 18°................	8.8 per cent alcohol.
At 9°................	9.5 per cent alcohol.

Concerning the lactic fermentation some investigators find no difference in the end-point, while others obtained results similar to the results with alcohol. With three strains of *Bact. lactis acidi* were obtained after thirty-four days, by C. W. Brown:

	A	B	C	
At 37°	0.89 per cent	0.87 per cent	0.60 per cent	of lactic acid.
At 30°	1.00 per cent	0.96 per cent	0.81 per cent	of lactic acid.
At 18°	1.08 per cent	1.06 per cent	0.88 per cent	of lactic acid.
At 6°	0.70 per cent	0.73 per cent	0.62 per cent	of lactic acid.

These results are quite logical and perhaps can be explained by the recognized experience that all products of fermentation tend to check the process of fermentation, and that any chemical product or substance acts the more vigorously upon any life process the higher the temperature. The same amount of alcohol that will still allow a slow fermentation at 10° may check the fermentation entirely at 20° Naturally the rate of fermentation in the beginning will be higher at the higher temperature but the end-point is lower. The end-point of the lactic cultures A, B, and C at 6° is probably not final, because thirty-four days is a short time of growth at so low a temperature. Above the optimum, the rate of decomposition will decrease rapidly with the rising temperature and the end-point will also be lower.

Freezing.—The discussion of the relation of temperature to microörganisms has so far considered only the temperatures within the limits of growth. However, the temperatures below the minimum and above the maximum are also of greatest importance. If bacteria are cooled below their minimum temperature they do not die immediately. They remain alive in a dormant condition ready to multiply as soon as the temperature rises. Even the freezing of a liquid will not kill them immediately. Of course, they cannot multiply in ice, because they have no water, consequently no food, and they cannot thaw the ice to get their water and food for lack of body temperature of their own. As long as liquids are frozen solid the bacteria in them will remain dormant much like dried organisms, and like them their number will decrease very slowly. An example is given in the following table relevant to the number of bacteria in frozen milk (after Bischoff). The decrease in numbers is not very uniform, since there are many different bacteria in milk, but the general tendency is the same as in the dried bacteria.

Milk kept at 3° to −7°

Freshly frozen	200,000 bacteria per c.c.
After 1 day	105,500 bacteria per c.c.
After 2 days	72,300 bacteria per c.c.
After 3 days	62,000 bacteria per c.c.
After 4 days	46,400 bacteria per c.c.
After 7 days	44,000 bacteria per c.c.
After 14 days	40,500 bacteria per c.c.
After 21 days	30,300 bacteria per c.c.
After 35 days	22,500 bacteria per c.c.
After 49 days	14,200 bacteria per c.c.

The table shows plainly that it is impossible to sterilize milk by freezing, but as long as it is frozen it will keep; there is no possibility of any microörganisms decomposing a frozen liquid, for the organisms need water above all. If food substances change in cold storage (and some food products do deteriorate), this must either be due to changes other than microbial or the material was not completely frozen as is probably the case with salted butter.

After bacteria are once frozen, they do not seem to be affected by any lower temperature. Macfadyen and Rowland found that they tolerate very low temperatures remarkably well. Many bacteria were not killed by a twenty hours' exposure to the temperature of liquid hydrogen (−252°). Yeasts are not quite so resistant and the mycelium of most molds is easily destroyed by freezing, while the spores are hardier.

Thermal Death-point.—Heating above the maximum temperature is quite harmful to bacteria, and the amount of injury increases with the temperature. Recent experiments have shown that heat does not kill bacteria instantaneously, but that we have an orderly process as in the case of death by drying. This can be observed only in a very narrow range of temperature, however, since the death rate rises very rapidly with the increase of temperature. 10° increase may make the death rate ten to one hundred times as great, and death is almost instantaneous. For most practical purposes, it is sufficient to state the time and temperature necessary to bring about complete sterilization. It has become customary to define, as the thermal death-point, the lowest temperature at which a culture will be killed in ten minutes. As most bacteriologists will use very nearly the same technic, they will have fairly uniform numbers of cells to start with, and therefore obtain fairly uniform results.

The thermal death-point does not depend upon the species and the temperature only. It varies with the age of the culture since older cells are less resistant than younger ones especially if heated in their own products. The medium in which the organisms are heated is also of great significance. The fact that acid liquids, as fruit juices, are more easily sterilized than neutral meat or vegetables is largely due to a chemical (poisonous) action of the acids upon the bacteria. But the greater resistance of tubercle bacteria in the sputum compared with those suspended in salt solution cannot be so readily accounted for.

A necessary factor for the prompt destruction of organisms by heat is the presence of moisture. The resistance of dry organisms is remarkably higher than that of the same organisms in a liquid culture. The following table shows the death-point of yeast cells and spores in a dry and moist state.

THERMAL DEATH-POINT OF DRY AND MOIST YEAST

Variety of yeast	Cells		Spores	
	Moist	Dry	Moist	Dry
Pale ale yeast	65°	95°–105°	65°–70°	115°–125°
Hofbräu yeast	55°	85°– 90°	65°	115°–120°
Saccharomyces pasteurianus	50°–55°	100°–105°	60°	115°

RESISTANCE OF SPORES.—The organisms most resistant to heat are the spores of certain bacteria. In the chapter on moisture requirements attention has been called to the great resistance of spores to drying. We find the same exceptional resistance to high temperatures. Boiling heat will not kill spores readily. Some bacterial spores can stand the temperature of 100° for several hours. In order to kill spores in one heating the temperature must rise to about 110° for fifteen to thirty minutes; this can be accomplished only by heating under pressure. This is not always advisable for sterilizing food substances. While vegetables are usually sterilized under pressure without losing much of their palatability, other foods like milk are changed materially in taste and appearance. To prevent these changes, discontinuous sterilization is sometimes used. This is based upon the following principle.

If milk or any other medium is heated to 100° for about fifteen minutes, all living cells of bacteria, yeasts and molds will be killed except a few spores of bacteria. After cooling, these spores will germinate under suitable conditions and the vegetative cells thus appearing instead of the resistant spores are easily killed in a second heating. A third heating is necessary in order to kill any vegetative cells which may have developed from spores not yet germinated before the second heating. It is essential to have the time between two heatings long enough to allow the germination of spores, and not too long to permit formation of new spores. It is customary to heat on three successive days for fifteen minutes each time. In this case, sterilization is usually complete, while a forty-five minutes' heating at once is not sufficient to guarantee sterilization. Among the substances that are very easily sterilized are cider and other fruit juices, while milk and soil are the most difficult materials to sterilize.

Dry spores will resist still higher temperatures than moist spores. Some dry spores survive an exposure to 140° or 150° for ten minutes. It requires a very high temperature to sterilize glass, cotton, gauze, and instruments with dry heat. A discontinuous sterilization of dry material is useless, since the spores will not germinate without moisture, therefore their resistance remains unaltered.

The spores of molds are more resistant than the mycelium, but if moist, they all die at 100°. The dry mold spores can tolerate a somewhat higher temperature, but not as high as the spores of many bacteria. Yeast spores and yeast cells are very much alike in their resistance to heat. The table on page 276 shows hardly any difference between their resistance.

CHAPTER III

INFLUENCE OF LIGHT AND OTHER RAYS*

Microörganisms in their natural environment are temporarily but not usually exposed to light. The organisms of decay, living in soil, in foods, in the intestines of animals, will only occasionally come in contact with the direct rays of the sun. Water bacteria and the organisms on the surface of plants and animals are more commonly exposed to the sun.

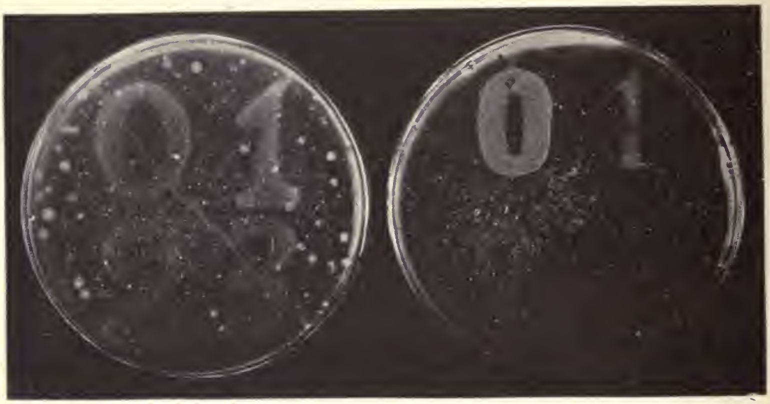

Fig. 117.—These plates were heavily inoculated with *B. coli* and *B. prodigiosus* respectively and then were exposed, bottom side up, to the direct rays of the sun, for four hours. On the instant of exposure, a figure O cut from black paper was pasted to the plate shading the bacteria underneath. After one, two and three hours the corresponding figures were pasted to the plates. The above picture was taken 24 hours after exposure, proving that three or four hours of direct sunlight weaken and and may even kill bacteria. *B. prodigiosus* proved more sensitive than *B. coli.* (*Original.*)

The influence of light varies with its intensity. Direct sunlight has a very harmful effect upon microörganisms. Most bacteria are killed by direct sunlight in a few hours; the time depends upon the organism as well as upon the intensity of light; this again varies with the amount of moisture and dust in the atmosphere, with the time of the day and with the season; an absolute measure for the action of light cannot be fixed, therefore, as easily as with the action of heat in the thermal death-point. The different colors of the spectrum do not act alike; the part of the spectrum from red to green is practically without influence upon microörganisms, while the blue light acts strongest and the intensity decreases in the violet and ultra-violet. In carrying on experiments with the influence of light, it must be remembered that glass absorbs ultra-violet rays, and further that the heating of the medium by direct radiation must be avoided (Fig. 117).

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* Prepared by Otto Rahn.

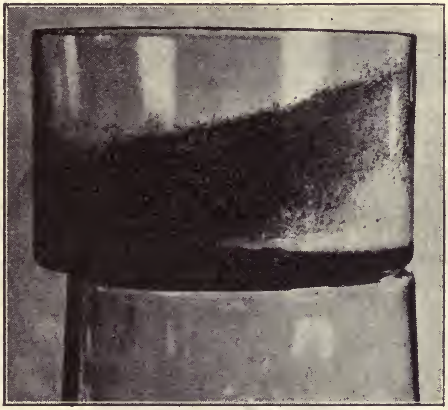

FIG. 118.—Phototropism of *Rhizopus nigricans*. The mold is grown on gelatin with diffused light coming from right side. (*Original.*)

Yeasts, molds, and bacteria and probably *Protozoa* are equally sensitive to light. Even the spores of most bacteria do not show a greater resistance to light, while the mold spores are an exception. The colored spores of the *Penicillium*, *Aspergillus* and *Mucor* species can be exposed to light for a long time without being killed, but the colorless spores of *Oidium* and *Chalara* show no increased resistance. It is supposed that the pigment in mold spores is a protection against light. This is not true with the pigment of bacteria. The colored and colorless strains of pigmented bacteria show no difference in their resistance to light. The only exceptions are the so-called purple bacteria. These peculiar organisms, many of which feed on hydrogen sulphide, seem to thrive better in light than without it. Direct sunlight does not kill them, it rather attracts them and they move toward the light. This is called *phototaxis* or *heliotaxis*. The pigment, bacteriopurpurin, does not take the place of chlorophyl, however, since the bacteria do not produce oxygen in light and always need organic food.

The effect of light upon microörganisms is mainly brought about by a chemical change in the protoplasm, and also, to some extent, by a chemical change in the medium, namely the formation of a peroxide or a similar oxidizing agent.

The germicidal action of light is of importance in the purification of rivers. It is applied also in curing diseases of the skin, as lupus and leprosy, by exposing the diseased parts to a very concentrated light of the electric arc. This light contains plenty of blue and violet rays and is preferable to sunlight because it is always ready for use and its composition and intensity can be controlled easily. Ultra-violet light is used in the sterilization of water and of milk.

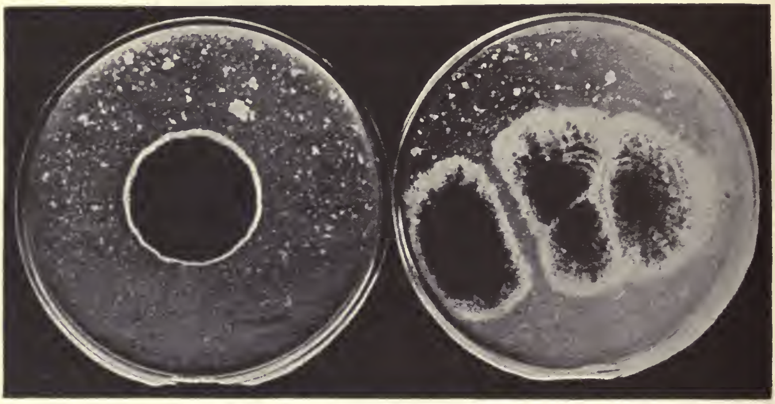

FIG. 119.—Two cultures of an *Aspergillus*, one grown in the dark the other in diffused light, showing rings. (*Original.*)

Diffuse light is not nearly as harmful to microörganisms as direct sunlight. Long exposures to diffuse light will kill most bacteria, while molds are not at all sensitive. They rather like a very dim light, and many molds grown in a dark room with light only from one side will grow toward the light. This property, which is characteristic for all green plants, is called *heliotropism* or *phototropism* (Fig. 118). It has been found that molds produce mycelium mostly in the dark, while in daylight sporangia are produced mainly. This difference in the development during the day and during the night accounts for the concentric rings which are quite commonly found in older mold colonies, and which indicate the age of the culture (Fig. 119). Similar rings are occasionally found with yeast and bacterial colonies, and are possibly due to the same influence of light.

X-RAYS.—Of other rays, the invisible X-rays and the radium rays have attracted the attention of bacteriologists and physiologists. It is known that the X-rays will destroy living tissue by long exposures; microörganisms cannot be considered less resistant. X-rays are used in the treatment of microbial diseases of the scalp and skin.

RADIUM RAYS are not so well known, and their bactericidal action is doubtful. The treatment of certain bacterial diseases has been attempted, but it has not been applied as generally as yet as the X-ray method. The sterilization of milk and possibly other foods by this method has been suggested, but the practical application is at present quite improbable because of the cost and the uncertainty of the results.

CHAPTER IV

INFLUENCE OF ELECTRICITY*

The influence of electricity upon microörganisms is much less than one might perhaps expect, if the electricity as such is considered. A direct electric current passing through a nutrient medium will, of course, cause electrolysis which is usually manifested by the formation of acid on the positive pole and of alkali on the negative pole. The acid and alkali will kill microörganisms, as is discussed in the chapter on chemical influences. In this case, it is not the electricity itself that destroys the bacteria. It is also possible to kill bacterial cultures by passing an alternating current through the medium for some time. No electrolysis takes place in this case, still it is not the current that acts directly upon the organisms, but rather the heat produced by the current passing through a medium of high resistance. If the culture is cooled properly the influence of the current is insignificant if at all noticeable. Whenever electricity is applied against microörganisms the effect is considered electrochemical.

The electrical current is used in a very small way in the purification of sewage. The sewage passes between two iron plates which represent the two poles of a strong current. The electrical sterilization of milk has been patented. Wines are improved by electricity. The sterilization of drinking water by ozone is also an application of electricity, though of course the ozone once formed by the current acts as a chemical compound independently of its source, and the same effect would be produced if the ozone were manufactured chemically.

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* Prepared by Otto Rahn.

CHAPTER V

INFLUENCE OF MECHANICAL AGENCIES*

Pressure.—The resistance of microörganisms to mechanical pressures is very great. Pressures of 3,000 atmospheres† will not kill the majority of bacteria in four hours. They are, however, weakened and some species will die. A specific difference between the molds, yeasts, and bacteria in this particular does not seem to exist. Of the organisms exposed to 2,000 atmospheres for ninety-six hours, *Bact. anthracis*, *Bact. pseudodiphtheriæ*, *M. pyogenes var. aureus*, *Oidium lactis* and *Saccharomyces cerevisiæ* survived, while seven other organisms lost the power of multiplication. Some of these were not dead, however, since they retained their motility for several days. It is noteworthy that high pressure will destroy one quality (multiplication) and not affect another (motility). Pigment-production and virulence of pathogenic bacteria were either diminished or lost completely. The resistance against high pressure is necessary for the organisms which cause the decay of organic matter at the bottom of the oceans. Vertebrates breathe oxygen in the form of gas or have at least an organ filled with gas (fish bladder); the volume of gas is changed considerably by slight changes of pressure; this will affect organisms depending on gas. Microörganisms do not require gas as such. They can absorb gases only in solution. A change of pressure therefore will not cause a change of volume, since liquids have a very small coefficient of compression.

The situation is entirely different if the liquid is not exposed to the pressure directly, but to compressed air. In this case, the chemical effect of the gas is the deciding agent. The higher the pressure, the more gas will be dissolved in the culture medium. The fatal pressure under these conditions will vary as much as the fatal dose of an antiseptic; it depends upon the chemical qualities of the gas, upon the pressure (concentration), upon the temperature, and upon the organism.

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* Prepared by Otto Rahn.

† One atmosphere is 1 kg. pressure per square centimeter (or about 15 pounds per square inch).

Some data have been given already in the chapter on oxygen requirements. It was mentioned in that connection that *Bact. butyricum* cannot tolerate more than 0.65 per cent of the total oxygen content in air (0.2 atmosphere); in other words, an oxygen pressure higher than 0.0013 atmosphere will kill the organism. The maximum pressure for *B. prodigiosus* was found to be about 5.4 to 6.3 atmospheres. Very few experiments have been made with other gases. Carbon dioxide at a pressure of 50 atmospheres retards the growth of bacteria in water and will sterilize it in twenty-four hours. Suspensions of pure cultures of *B. typhosus* and *Msp. comma* are killed by 50 atmospheres carbon dioxide pressure in three hours. Milk cannot be sterilized by this pressure but bacteria do not multiply. Carbonated milk has been recommended as a refreshing drink by several investigators. The ordinary market milk will keep about two days longer under the pressure of 10 atmospheres (150 pounds) than without pressure. If pasteurized it is said to keep for a week.

GRAVITY.—Gravity would have a great influence upon the growth of microörganisms in liquids if their specific gravity were much greater than that of water. This does not seem to be the case however. It has been estimated by accurate weighing to vary between 1.038 and 1.065. Very much higher results (1.3 to 1.5) have been obtained by centrifuging bacteria in salt solutions of varying specific gravity, but these data are not exact since the salt solution will diffuse into the cells and thus increase their weight. The specific gravity being very nearly that of the culture medium, it is plainly seen that gravity has but little influence. The microörganisms will live suspended in the liquid and sediment out very slowly. The slightest current in the liquid will carry them around and distribute them through the medium. The motility is of minor importance; the actual distance covered by motile bacteria has been measured, and under the most careful exclusion of currents in the liquid has been found to be about a millimeter in a minute for *B. subtilis*. This is very slow compared with the speed of the circulating water moved by changes of temperature or other incidental agents.

Yeast cells and other gas producers use the carbon dioxide as a vehicle. The gas bubbling up in the fermenting liquid keeps it constantly in motion and moves the yeast cells against gravity toward the surface where the gas escapes and lets the cells fall back to the bottom.

The production of scums and pellicles on the surface by organisms which are heavier than the liquid they float on, is often accomplished by small gas bubbles between the cells (*Mycodermæ*). In other instances, it may be just the floating of cells having oily surfaces.

The growth is influenced by gravity very little. The sporangia of molds are the only exceptions, growing decidedly away from the center of gravity (*negative geotropism*).

AGITATION.—For the majority of microörganisms, the quiet, undisturbed growth of the laboratory culture is the normal or the ideal one. Such cultures, if shaken for a considerable time, show a decrease of living organisms, and it is possible to sterilize cultures by continued shaking. The effect is not a simple mechanical breaking or tearing of the cells. The bacteria break up into the finest particles. This is also the case if cultures are exposed for several days to the trembling motion caused by the working of very heavy machines. There is no grinding or tearing effect but the cells break to pieces just the same.

A slight and slow agitation seems to be advantageous for many cultures, only continuous heavy motion proves harmful. Different organisms show wide variations in their resistance to agitation.

DIVISION IV

CHEMICAL INFLUENCES

CHAPTER I

STIMULATION OF GROWTH*

The influence of chemical substances upon microörganisms may be helpful or harmful, or not noticeable. As helpful must be considered above all the food compounds. Unless given in such large doses as to cause a physical or osmotic effect they will stimulate the development. Other substances, not food, can also act as stimulants. It is a recognized fact of long standing that many poisons in very small doses will stimulate. This applies to the most highly developed animals and plants as well as to microorganisms. Raulin noticed in 1869 that *Aspergillus niger* grew very much better in a nutrient solution if a small amount of zinc salt was added. He considered the zinc, therefore, as a necessary constituent of the mold cells. Alcoholic fermentation can be stimulated by metallic salts. It is believed by some physiologists that, as a law of nature, every substance that is injurious in a certain concentration is a stimulant in a lower concentration. A similar action of certain chemical compounds upon enzymes has been noticed, retarding in high concentrations, stimulating in weaker solution.

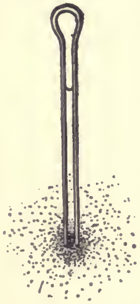

FIG. 120.—Chemotaxis. (*After Fischer.*)

CHEMOTROPISM AND CHEMOTAXIS.—Microörganisms manifest their preference for certain foods not by a stimulated growth alone. They also make efforts to obtain better food by growing or moving toward it, which is not a manifestation of a rudimentary intellect. Such reactions of microörganisms may be accounted for largely by chemical or osmotic forces. In a solid medium the hyphæ of molds will grow toward the best source of food supply. This growth on account of chemical stimulation is called *chemotropism*, analogous to the *phototropism* or growth toward light. If some injurious compound is offered, the hyphæ will grow away from it. Thus we have to distinguish between *positive* and *negative chemotropism*. The motile organisms, bacteria as well as protozoa, demonstrate their preference for certain food compounds by swimming toward them. This is called *chemotaxis* (Fig. 120). Here also a *positive* and *negative chemotaxis* must be distinguished, the latter taking place if injurious substances are present.

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* Prepared by Otto Rahn.

CHAPTER II

INHIBITION OF GROWTH*

Poisons, Germicides, Disinfectants, Antiseptics, Preservatives

A great number of inorganic and organic bodies will destroy life in comparatively weak solutions. These substances are called *poisons* if they are considered in their effect upon man and animals. In their application to microörganisms they are generally called *germicides* (germ-killers), or *disinfectants* if the emphasis is laid upon the prevention of infection rather than upon the actual killing of the microörganisms. Analogous to the general term germicides, the terms *bactericide* and *fungicide* are used occasionally. The term *antiseptic* means a prevention of sepsis which may be accomplished by checking the growth without necessarily killing all microörganisms. The meaning of the word *preservative* is practically the same, only the latter is used more commonly in relation to foods, feeding stuffs and preparations of similar origin while the word *antiseptic* is largely used in relation to microbial diseases. A strict line cannot be drawn between any of these definitions. A disinfectant, if diluted, becomes an antiseptic. A strong salt solution is an antiseptic for some organisms and a disinfectant for others. Of the above expressions, germicide is the most definite, but is not so commonly used as the others.

Mode of Action.—The action of a poison upon the cell is generally considered an action upon the protoplasm. The poison is supposed to combine chemically with the cell plasma producing compounds which interfere with the continuation of the life processes and thus cause death. If the cell has been subjected to the action of the poison only a short time, it can be saved by removing the poison. Bacteria can be treated with mercuric chloride ($HgCl_2$) so that they will no longer develop if transferred to a fresh medium. If the mercuric chloride is removed from the cell by means of hydrogen sulphide, some of the organisms may be revived.

The mode of death through poison is the same as that through heat or drying. The number of cells dying in a given time interval is proportional to the number of cells surviving. In the last five years, this has been tested and found true with practically all disinfectants. Fig. 121 shows the curves plotted from data obtained with *Bact. anthracis*, the full-drawn line representing the number of live spores in .21 per cent of mercuric bichloride, the dotted line the same in .11 per cent solution.

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* Prepared by Otto Rahn.

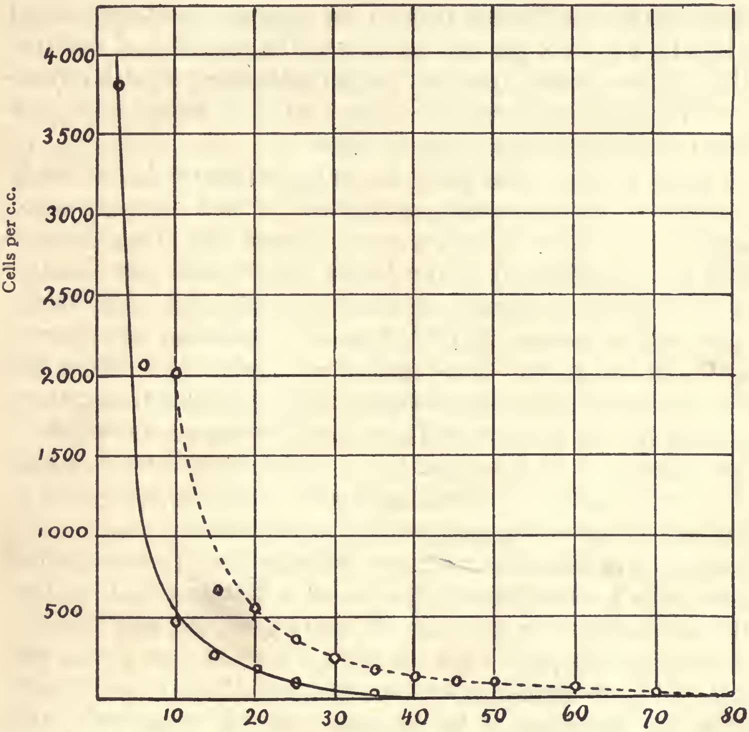

FIG. 121—Curve of disinfection. Spores of *Bact. anthracis* in mercuric chloride solution. (*After Chick.*)

The (apparent) resistance of the few remaining cells is of great importance in those applications of disinfection where a thorough killing of all bacteria is intended, *e.g.*, in the treatment of drinking water. Our ideas of the efficiency of a disinfectant would depend, therefore, upon the accuracy with which we can prove the presence of a certain bacterium.

Factors Influencing Disinfection.—The efficiency of a disinfectant depends upon several factors. Moisture is necessary—a dry poison has only a very slow action upon microörganisms. For this reason, absolute alcohol has not nearly the same germicidal power upon dry bacteria as diluted alcohol; the strongest poisonous effect is obtained by a 50 to 70 per cent solution. The necessity of moisture is further demonstrated in the sterilization with gases, as with formaldehyde. The effect of formaldehyde gas without the provision of a very moist atmosphere is surprisingly weak.

The temperature is also quite an important factor in the study of disinfectants. Since poisoning is supposed to be a chemical effect, it must be expected that the poisoning process like other chemical processes will take place faster at a higher temperature. As a matter of fact, the death rate through poisoning is usually doubled or trebled by a temperature increase of 10°. Above the optimum temperature, where the growth is not very vigorous, and when the disinfecting power of the poison is increased considerably by the higher temperature, a very small amount of poison will have a very strong germicidal effect. The combination of high temperatures with a disinfectant has been suggested as a means of sterilizing foods. This has been tried in the case of milk with hydrogen peroxide at 50° to 60°.

It makes a considerable difference whether the organisms which are tested with a certain disinfectant are in a culture with their food material, or suspended in water or salt solution without any food. It is very probable that part of the disinfectant is acted upon by the food products which are partly protein substances and are in many ways similar to the protoplasm of the bacterial cells. It is especially difficult to poison bacteria in blood, pus, or similar material. The sensibility of the microörganisms in pure water is remarkable. Very small doses which would not be considered efficient under any other condition, will destroy microörganisms in pure water. The concentration of chloride of lime which is sufficient to sterilize drinking water, does not at all suppress the development of bacteria in sewage.

The influence of the number of cells is evident from the above explanations of the mode of action, and from the curves of disinfection. The concentration of the poison is of course of greatest importance.

Recent investigations have shown the rather unexpected fact that the efficiency of a poison is not proportional to its concentration. If a certain poisonous solution is diluted with an equal volume of water, we might expect it to be half as poisonous as before, but depending upon the chemical nature of the poison, it may be more poisonous than expected, or considerably less. It follows from this that two different poisons of the same intensity, if diluted in the same proportion, may not have the same intensity any more.

Microörganisms will gradually become accustomed to certain poisons, and become more resistant. This principle has been utilized in the manufacture of distilled alcohol; yeasts have been cultivated which can tolerate a high concentration of acid; the acid serves to suppress bacteria producing undesirable fermentations.

The age of the culture and the stage of development will naturally change the resistance of a species materially. The old cultures which are past the culmination of growth will be much more sensitive to any poison unless a spore-producing organism is under test. In this case, we find a greatly increased resistance, similar to the increased resistance of spores against drying and heat.

THE CLASSIFICATION OF DISINFECTANTS is very difficult as long as we cannot explain completely the process of poisoning. It is impossible to arrange them according to the intensity of action, because the intensity of influence depends not only upon the disinfectant, but also upon the species of organisms. Some yeasts can resist ten times as much alcohol as certain bacteria. Formaldehyde is not nearly as strong an agent with molds as it is with bacteria. The disinfectant concentration of a poisonous substance is not absolute. The simplest method of grouping is by chemical structure and qualities. Of the following natural groups can be distinguished acids (inorganic and organic), metallic salts, hydrocarbons (aliphatic and cyclic), alcohols (aliphatic and cyclic), aldehydes, anæsthetics, essential oils, oxidizing agents and reducing agents.

The first three groups, acids, alkalies and salts, are distinguished from the rest as electrolytes; the strength of acids and alkalies (chemically speaking) is measured by the degree of electrolytic dissociation. The disinfectant value follows largely the same law. The strongest acids in the chemical sense are also the strongest disinfectants. There are exceptions, however, where, besides the poisonous effect due to the degree of dissociation, there is a specific effect due to the chemical structure, as is the case of nitrous, salicylic and hydrocyanic acids. The same is true of alkalies. With metallic salts, the action will depend mainly upon the metal in solution, but the electrolytic dissociation is also of importance. NaCl will decrease the dissociation of mercuric chloride ($HgCl_2$) and decrease also its disinfectant power. Mercuric chloride dissolved in absolute alcohol is not dissociated. In this case, it has almost no action upon bacteria.

Acids are not commonly used as disinfectants, except in the household, but they play a certain rôle in nature. The common fruits contain so much acid that bacteria cannot easily attack them; the decaying of fruit is almost exclusively due to molds which have a preference for acid media. The acid in the stomach of man and animals plays an important rôle as a sterilizing agent for the food. Many microörganisms are killed in the stomach. In the household, the natural acidity of fruit helps in keeping canned fruit, preserves and jellies. Especially in heating, the acid together with the high temperature has a very strong germicidal effect. Vinegar is often used to preserve fruit and vegetables; in some parts of the country, meat is kept in buttermilk. Benzoic and salicylic acids are often used in the preservation of fruit and vegetables. Their poisonous influence is not so much due to the acid reaction but to the specific chemical character of these compounds.

Of the alkalies, only one is used extensively, namely, lime; quicklime (CaO) is considered a valuable disinfectant for excreta in privy vaults; it is universally applied as a whitewash in stables, barns, poultry houses and similar buildings. Quite commonly, it is used as "milk of lime" (one part of slaked lime with four parts of water). It should be kept in mind that the calcium oxide unites with the carbon dioxide of the air and thus gradually loses its disinfecting power.

Of the metallic salts, many are well-known germicides. The most powerful disinfectant is mercuric chloride ($HgCl_2$) which is one of the standard disinfectants. It is generally used in a dilution 1 : 1000 which is sufficient to kill all vegetative cells as well as spores in a few minutes. Quite commonly, hydrochloric acid or salt is added, to prevent coagulation or precipitation of slimy or albuminous matter which would protect the enclosed bacteria from immediate contact with the poison. The addition of hydrochloric acid or any chloride decreases somewhat the disinfectant value for bacteria suspended in distilled water because it decreases the electrolytic dissociation.

Another disinfectant of remarkable strength is silver nitrate; it is not used commonly because of its high price. It also decomposes easily and leaves dark spots on the skin and clothes. Of the other metallic salts, copper and iron sulphate are not used extensively, though recommended for the disinfection of feces. Zinc sulphate may be applied to mucous membrane the same as silver nitrate. Many other salts may be used occasionally for disinfecting purposes, though the expense or undesirable qualities prevent their common application.

The alcohols are well known for their poisonous effects, but the value of ethyl alcohol as a disinfectant is usually overestimated. It takes quite strong alcoholic solutions, more than 20 per cent, to kill certain yeasts and the spores of some bacteria in less than a day, and a complete sterilization by alcohol in a few minutes cannot always be guaranteed even with 50 to 60 per cent solution. It has already been mentioned that desiccated organisms are very resistant to concentrated alcohol, more so than to a 50 per cent mixture. Methyl alcohol is weaker, the higher alcohols, especially amyl alcohol, are stronger disinfectants than ethyl alcohol. They all give good results in the presence of water while the absolute alcohols have scarcely any effect upon desiccated bacteria. None of these alcohols in whatever concentration they may be used, can be relied upon to kill bacterial spores.

Stronger germicidal effects can be obtained by the alcohols of the benzol group, of which phenol or so-called carbolic acid (C_6H_5OH) is the simplest representative. Phenol, like ethyl alcohol, is not as effective as is commonly believed. It is applied in solutions from .5 per cent to 5 per cent ordinarily, but it usually takes a long time even for the 5 per cent solution to kill vegetative cells as *Bact. tuberculosis* or *B. coli;* it is inefficient against anthrax spores. More powerful are the higher cyclic alcohols, of which the cresols are examples. They are used extensively as disinfectants and antiseptics. They are, together with phenol, coal-tar constituents and are sold commercially under many different names either pure or mixed with soap or other disinfectants which make them emulsify readily in water. The cresols are almost insoluble in water, and not as effective in solutions as they are in emulsions. The disinfecting properties of tar come from the cresol contained in it.

Hydrocarbons are used only for laboratory experiments as very weak antiseptics. The aliphatic bodies, as methane, etc., which constitute a large part of coal gas, have very little if any effect upon bacteria; gas is used occasionally in place of hydrogen for growing anaerobic bacteria. Benzol, xylol, and toluol are antiseptics, if shaken frequently with the liquid to be protected, but they are not reliable as disinfectants. The same is true with the common anæsthetics, ether and chloroform. The high prices of these agents forbid their general use, but they are sometimes used for laboratory work.

The essential oils have a little more practical importance. Some of these are the main constituents of mouth washes, especially the oil of peppermint (menthol), of thyme (thymol), and of eucalyptus (eucalyptol). Their action is very weak, however. The volatile oils of spices have to be considered in the preserving of fruit, pickles, catsups, and other food products. Though the antiseptic value in general is insignificant, certain microörganisms are sensitive to certain spices. The bacteria of the mesentericus group are said to be suppressed entirely by quite small quantities of garlic, while others, like the lactic bacteria, are not affected at all. Cloves, cinnamon and allspice are the most efficient spices, while the disinfectant power of black and white pepper and mustard is very small.

The most important disinfectant has not been mentioned, because it does not belong to any of the above groups. This is formaldehyde. Formaldehyde ($HCOH$) is a gas, soluble in water to the amount of 40 per cent at room temperature; it does not attack metal, clothing, woodwork, and is, therefore, preferable to many other disinfectants for sterilizing rooms. It kills spores of bacteria in a short time in a 1 : 1000 dilution. Its greatest importance lies, however, in its gaseous nature, because it can be applied to rooms and buildings by simply evaporating it. The saturated 40 per cent solution can be evaporated directly or by generating steam which passes through the formaldehyde solution; this latter method has the advantage of saturating the air with moisture, which increases the power of the formaldehyde gas. Formaldehyde can also be obtained in a dry form; it polymerizes to a white crystalline substance, paraformaldehyde ($(HCOH)_3$) which can be changed back to formaldehyde gas by gentle heating. This paraformaldehyde is commonly used instead of the liquid, because it is more easily handled and is quite inoffensive in its solid form, while the formaldehyde solution has a very penetrating odor and is exceedingly harmful to the mucous membrane of the respiratory organs.

Of the oxidizing agents, oxygen itself has already been mentioned. Though it is able to destroy certain anaerobic bacteria, it cannot be called a disinfectant. For this purpose, oxygen must be activated; such oxygen can be obtained in the form of ozone (O_3). It is formed in air under the influence of electric discharges and can be produced at a price low enough to allow its application for use in the sterilization of water. It has also been recommended for preservation of milk.

Hydrogen peroxide (H_2O_2) resembles ozone in its chemical reactions; it changes readily to $H_2O + O$, and this oxygen atom in the nascent state is quite effective as an oxidizing agent. For an antiseptic, it must be used in at least a 1 per cent solution, and for an absolutely reliable disinfectant a still higher concentration is required. It loses its disinfecting property easily because it is decomposed readily by the peroxidases of tissues and organic liquids as blood, milk, and pus. It is used in the preservation of milk. Hydrogen peroxide is slowly decomposed by the katalase of milk thus disappearing completely.

Chlorine in its gaseous form is not used as a disinfectant, though its germicidal power is quite strong. The so-called "chloride of lime," manufactured by absorbing chlorine in slaked lime, gives in water hypochlorite and free chlorine; these substances are good germicides and chloride of lime is used in the disinfection of privy vaults, and other places in which it may be employed without injury. Hypochlorite is now used with great success for rendering safe drinking water and sewage; it has also become the basis of some commercial disinfectants.

Potassium permanganate is only incidentally used as a disinfectant. Its chemical qualities prevent an ordinary use.

Sulphurous acid, or sulphur dioxide (SO_2) was for a long time a standard disinfectant and is still used occasionally for fumigating rooms, stables, barns and out-buildings though it is substituted more and more by formaldehyde which can be applied almost as easily. The burning of sulphur is an extremely simple process, but it requires a moist air to disinfect properly, and under these circumstances it will attack metal, dyes of clothing and even the fiber itself.

In addition to these disinfectants which are used outside of the human body, or applied to its surface only, there have come into use during recent years, several disinfectants which are injected into the body to kill the microörganisms in the blood. Among these might be mentioned the colloidal metals, mainly colloidal silver which is sold under various trade names, *e.g.*, collargol. It is given especially in pneumonia, but its action upon the bacteria directly is very insignificant, though it greatly stimulates phagocytosis. Further, there is to be mentioned ethoxyl, given against the protozoön of sleeping sickness, and the latest and most discussed of all, salvarsan, an organic arsene compound, against syphilis.

DIVISION V

MUTUAL INFLUENCES*

INTRODUCTION

The biological relations of microörganisms are of the greatest importance in nature. Pure cultures in nature are very rare and of exceptional occurrence; they are hardly ever found except in certain diseases of man, animals and plants. Generally, nature works with mixed cultures. All natural fermentations, decompositions and putrefractions are accomplished by a number of different species among which perhaps one dominates, but is influenced by the rest. The study of the mutual relations of microörganisms is in the very first stage as yet; practically all laboratory work is done with pure cultures. The experiences obtained with pure cultures are not sufficient to explain all microbial activity in nature.

There are many possibilities of mutual influence between differen. organisms. Generally three main cases are distinguished: *symbiosis*, where two organisms profit by the combination; *metabiosis*, where one profits by the other's action without benefiting the other in return, and *antibiosis*, where one organism injures the other. These cases cannot be separated strictly. The relations are not always constant through the entire development of the cultures; an originally beneficial influence may change to an injurious one in a few days. Many terms have been coined to designate all these various possibilities, but in order to avoid this multiplicity of more or less indefinite names for the various relations, the general term "association" has come into use, especially when the relationship is not well understood.

SYMBIOSIS

Symbiosis is not very common among microörganisms, and it is difficult to find examples where true symbiosis exists through the entire development of both organisms. The association of lactic bacteria and *Oidium lactis* in milk is, for a certain period at least, a symbiosis. The bacterium will produce only a certain amount of acid, and then it can grow no more because the acid is too strong; the mold will destroy the acid and thus gives the bacterium a chance for continued activity. The bacterium produces the acid which the mold likes; the mold in turn removes the excess acid which otherwise would check the bacterial activity.

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* Prepared by Otto Rahn.

True symbiosis is more common in the relation of microörganisms with higher plants and animals. The standard example in the plant kingdom is *Ps. radicicola* in the nodules of legumes, feeding on carbohydrates provided by the plant and furnishing the plant nitrogen from the air which the plant cannot assimilate directly. The typical example in the animal kingdom is *B. coli* in the intestine of animals, being nourished by the food of the animal and rendering the food more easily digestible.

METABIOSIS

Metabiosis may be considered a one-sided symbiosis; two organisms live together, but only one is benefited, the other remains uninfluenced or later may be injured by the association; the latter case is the most common. In this relation, one usually prepares the food for the other. It has previously been mentioned that the metabolic products of one species serve as food for another species, thus breaking up the various organic compounds step by step to smaller and simpler molecules. Quite commonly, each step is accomplished by a different species of microörganism. Consequently, metabiosis is a very common occurrence among microörganisms.

The classical example is the two nitrifying bacteria: the nitrate bacterium is unable to oxidize ammonia, and depends entirely upon the nitrite bacterium to oxidize the ammonia to nitrite; then, and only then, can the nitrite bacterium grow.

The relation between yeasts and acetic bacteria is also very well known. The yeast ferments the sugar to alcohol, and then the acetic organisms oxidize the alcohol to acetic acid. The yeast is in no way helped by the acetic bacteria, while these could not form acetic acid from sugar readily. These bacteria depend upon the action of the alcohol-forming yeast. Other cases of metabiosis are found in the association of lactic bacteria with certain protein destroying organisms. The lactic bacteria often develop much better if the protein bacteria grow together with them or have grown previously in milk. Metabiosis does not require the growth of the two associated organisms at the same time. The effect will be the same if first the one and later the other develops, and even after the first organism is killed or removed, its effect upon the pure culture of the second will still be noticed. This does not occur in the case of symbiosis.

One species can favor the development of another by other means than food provision or preparation. Certain bacteria cannot live in acid media, and molds or mycodermas destroying the acid will render possible the growth of these bacteria though they do not provide them with food. This is the case in the ripening of certain soft cheeses. Another example is the production of heat by fermenting organisms in manure, hay, ensilage, enabling the development of thermophile organisms. A very interesting and important problem is the growth of strictly anaerobic bacteria near the surface of liquids in association with some aerobic bacteria. How this is really possible cannot be satisfactorily explained. Though the aerobic bacteria continuously remove the oxygen from the water a certain amount will remain, sufficient to prevent the growth of the anaerobic bacteria under ordinary conditions. There seems to be a certain protective influence derived from the aerobic bacteria, the nature of which is unknown.

ANTIBIOSIS

The standard examples of antibiosis are the alcohol production by yeast in sugar solutions and the acid production by lactic bacteria in milk. Fresh cider contains a large number of bacteria, yeasts and molds; some of these organisms cannot develop in the acid medium, but many will begin to grow. Some of the bacteria will produce or destroy acid, others may begin to work on the nitrogenous material of the cider, and the yeasts produce alcohol and carbon dioxide. The carbon dioxide will soon saturate the cider and begin to bubble up, thus removing the other gases. The molds will stop growing if the oxygen is taken away, but some of the bacteria may continue growing until the alcohol concentration checks their further development. They first cease to grow, then cease to produce acid and finally die, while the yeast is still continuing in the fermentation.

In the lactic fermentation of milk, *Bact. lactis acidi* combats all other organisms by a rapid production of lactic acid. Though it is present in fresh milk only in very small numbers, its rapid growth and the formation of acid which will check and even kill most other bacteria soon makes it the dominant organism in the flora of milk, and at the time of curdling, it is often difficult to find any other organisms besides the lactic bacteria. In the preceding chapter was mentioned the metabiosis of certain protein-digesting bacteria with *Bact. lactis acidi*. This metabiosis can be considered as such only from the standpoint of the lactic organism. The protein bacteria are killed by the acid formed by the rapidly growing lactic bacteria. From the viewpoint of the protein bacteria, the relation is antibiosis. Another illustration of antibiosis is the acetic fermentation. The formation of acetic acid prevents the development of all bacteria and of most yeasts and molds.

In all these cases, the deciding agent is a well-known chemical compound. In other combinations, the principle is unknown. *Bact. lactis acidi* will check the growth of *B. subtilis* not only in milk where it forms acid, but also in sugar-free broth where acid production is impossible. Acetic bacteria act upon the yeast cells not only by means of the acetic acid produced, but also by some other, unknown agent, since vinegar is more injurious than the corresponding amount of pure acetic acid in water. A very remarkable organism is *Ps. pyocyanea;* it secretes a substance, *pyocyanase*, which will kill and dissolve the cells of other bacteria rapidly.

Parasitism, which would be classified under antibiosis, has not been found to exist among bacteria or yeasts; but we know of cases where one mold grows on the other; this is especially true with the largest representatives of the mucor family, which are often attacked and sometimes killed by smaller fungi.

RELATIONS BETWEEN CELLS OF THE SAME SPECIES

That cells of the same species will also influence each other, may well be assumed. The simplest relation will be the competition for food. This will be the case in nature more commonly than in laboratory media which are, as a rule, so rich in nutrients that development ceases before all food is used up.

The cause for cessation of growth in a culture is of great theoretical and practical interest. Apparently there are various factors concerned in this. Lack of food; or of one single essential food compound, may be the cause. This is found sometimes in media where it would be least expected. Some strains of *Strept. lacticus* are supposedly limited in milk by the lack of available nitrogen; they cannot attack casein readily and albumin; besides these proteins, nitrogen compounds are not plentiful. Addition of peptone increases the maximum number of cells from 0.7 billion to 2.5 billions per c.c. More commonly, however, growth is checked by the accumulation of metabolic products. Yeasts are checked by the alcohol, and acid-formers by the acid, urea bacteria by the alkali. In many of these cases, the removal, or neutralization, of the inhibiting product will bring about new development.

The harmful products accumulating are not always of such simple nature. Some very interesting observations have been made during the last ten years. Eijkmann, as the first, found that *B. coli* reached its maximum growth in gelatin at 37° in a few days, and that this gelatin, after hardening at 20°, would not support growth after streaking with a young culture of the same organism; but after this gelatin had been heated at 60° for half an hour, *B. coli* grew on it as well as on fresh gelatin. Broth in which *B. coli* had grown became fit again for growth of the same bacillus after filtration through porcelain. The inhibition of growth is, in this case, due to a compound which resembles a toxin in many respects. The importance of such investigations to general physiology is evident.

PART III

APPLIED MICROBIOLOGY

DIVISION I*

MICROBIOLOGY OF AIR

CHAPTER I

THE MICROORGANISMS OF THE AIR AND THEIR DISTRIBUTION

The atmosphere is not the normal habitat of bacteria, for growth and multiplication cannot take place in it under ordinary conditions. The phrase "microörganisms of the air" is therefore somewhat ambiguous. The small size of microörganisms enables them to remain suspended for considerable periods when physical forces have separated them from the substrata on which they have developed.

MICROÖRGANISMS PRESENT IN THE AIR.—Molds, bacteria, and yeasts are all found in the air under certain conditions. The first two are usually relatively abundant, the latter are less common.

The common molds have adapted themselves for the most part to wind distribution. They bear spores that are small in size and possess a surface that is not readily moistened. These spores are resistant to desiccation and light and remain viable for a considerable time even under unfavorable conditions. Furthermore, the fruiting bodies of many, though not all molds, show a distinct negative hydrotropism, *i.e.*, the mycelium remains in contact with the moist substratum while the threads which bear the spores rise at right angles to it. These latter are so sensitive that they can detect slight differences in the moisture content of the air and grow in the direction which will bring the spores into the driest situations. A slight current of air will detach the spores from these structures and carry them long distances.

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* **Prepared by R. E. Buchanan.**

Bacteria and yeasts lack the specific adaptations for wind distribution found in molds. The material upon which they have been growing must be dried and pulverized before they can be blown about. Many species produce spores or other resistant cells, and physiologically are as well adapted for air distribution as are the molds.

OCCURRENCE IN THE AIR.—Microörganisms are found free in the air, attached to particles of dust, or enclosed in minute drops of water. Mold spores are commonly free or in unattached clusters. Bacteria and yeasts are usually associated with dust particles, frequently the pulverized substratum on which they have been growing. Not all dust particles have living organisms attached. It has been computed that in the air of London during a fog there is only one living organism for over thirty-eight millions of dust particles. Microörganisms are sometimes sprayed into the air with water. Droplets containing bacteria are thrown off in the saliva in coughing or in speaking, and from the surface of fermenting liquids on which bubbles are bursting. When the drop is small enough, the air currents keep it in suspension and the water soon evaporates and frees the organism. This brings about the condition first discussed, free bacteria in the air. The decrease in weight and size incident to this loss of water probably accounts for the fact that the so-called "infectious droplets" are sometimes carried for considerable distances.

HOW MICROÖRGANISMS ENTER THE AIR.—In comparatively few instances do microörganisms possess mechanical devices for projecting the spores or other cells into the air for wind distribution. Usually the organism is passive and is freed only by air currents or by mechanical agitation. Some molds, as has been stated, release their spores even in the presence of moisture, so that complete desiccation is unnecessary for their dispersal. Bacteria and yeasts, on the other hand, are not usually given off from moist surfaces. Only when dry and pulverized can the bacterial medium be readily blown about. Hansen found that in the immediate vicinity of a heap of decaying malt, the air was comparatively free from bacteria. Winslow has shown that sewer air is frequently practically free from bacteria although the surface with which it comes in contact teems with bacterial life. Mechanical agitation often throws large numbers of organisms into the air. Moving hay and straw, grooming animals, sweeping a floor or carpet will multiply the dust and bacterial content of the air many times. In a similar manner, tiny, germ-holding droplets may be scattered by the splashing of sewage or of fermenting or putrefying liquids, and in speaking, sneezing or coughing.

Conditions for Subsidence of Bacteria.—The length of time during which an organism may remain suspended in the air is dependent upon several factors. Small particles settle out more slowly than large for the reason that as the size of an object is decreased, the surface area decreases less rapidly, proportionately, than the volume. The lifting effect of air currents depends upon the ratio of surface area to volume and specific gravity. The smaller the object, therefore, the greater is the resistance to subsidence. Consequently, bacteria usually settle out of air very slowly if free in a quiet atmosphere. The time of suspension is determined also by the velocity of the air currents. While considerable velocity may be necessary to dislodge microörganisms and bring them into suspension, a very slight air current will sustain them. Winslow has found that a current of 17 inches per minute is sufficient to sustain *B. prodigiosus.* The relative humidity of the air is also an important factor. In a supersaturated air solid particles, such as bacteria, become foci of condensation for water and quickly settle out. When dust is present in considerable quantities, and certain electrical or moisture conditions exist, flocculation occurs and the larger bodies so formed subside rapidly. The character and abundance of surfaces with which the suspended particles may come in contact also play an important part. Moist surfaces are much more effective in retaining particles than those which are dry.

Determination of the Number of Bacteria in the Air.—The number of bacteria in the air is frequently determined by exposing open petri dishes of gelatin or agar in different places for definite periods. This is a comparative quantitative method only. The number of colonies developing upon these plates will give the number of dust particles having living spores or cells upon them that fall in the given area under the conditions of the experiment. Evidently this is of value only for rough comparative work as constantly shifting currents of air usually introduce great errors. A somewhat more accurate method is to draw measured volumes of air into a flask, the bottom of which is covered with a layer of gelatin or agar. The colonies which develop represent the number of organisms which settle out from the given volume. More accurate results still may be obtained by drawing measured volumes of air in small bubbles through liquid gelatin. Practically all of the particles will be retained and the number of colonies which develop may be counted. This method is sometimes modified by drawing the air through a definite volume of water, care being taken to insure sufficient contact of air and water to remove all dust particles. A proportionate part of the water is then plated and the number of organisms estimated. Air is sometimes drawn through a filter made of sugar, sodium sulphate, or sodium chloride, and this material then dissolved in water and plated. Sand, asbestos, glass, etc., are sometimes used as air filters, then thoroughly washed, and the wash water plated.

Relative quantitative examination of the air is of more historical than practical importance. It has been useful in the development of the germ theories of fermentation and of disease and in overthrowing the theory of spontaneous generation. There is so little ordinarily to be learned by a study of the air flora that a comparison of plates exposed directly will usually suffice. Where more accurate results are desired, one must resort to one of the filtration methods discussed above.

Qualitative determinations of the species of air organisms are not often made. When necessary it may be done by simple examination of the colonies developed on the plates or by animal inoculations made from the water used in the air filter. It is sometimes necessary to vary the composition of the medium used in order to favor the development of certain types of organisms desired, for example, a higher precentage of molds will be found and a more luxuriant development will take place if wort agar or acid gelatin is used.

Number of Bacteria in the Air.—The number of bacteria in the air is determined by a variety of conditions. The velocity of air currents and the nature of the surface with which these currents will come into contact, are probably most important. Bacteria are usually more abundant on quiet days in the air of buildings than out of doors, but on windy days the reverse is true. They are often more abundant in cities than in the country. Fewer are found at high altitudes and over large bodies of water. Frankland found that there are fewer in winter than in summer. They are washed from the air during rains. Bright sunlight destroys many. The nature of the soil and the vegetation covering it has a marked influence. The following figures from various authors are appended to serve as an index to what may be expected in the air content of bacteria.

Locality	Number of organisms per cubic meter	Observer
Outdoor air, Boston	100–150 bacteria. 50– 75 molds.	Sedgwick and Tucker.
Open air	100–150 bacteria.	Fischer.
Open field	250	Uffelman.
Seacoast	100	Uffelman.
Mountain altitude, 200 meters	0	Pasteur.
Mont Blanc	4– 11	Ellis.
Spitzbergen (Arctic Regions)	0	Levin.
Middle of Paris	4,000	Ellis.
Paris Street	3,500	Fischer.
Tailor's Room in Whitechapel	17,000	Ellis.
Boot Workshop	25,000	Ellis.

SPECIES OF ORGANISMS IN THE AIR.—*Penicillium* is the most common mold isolated from the air. Next in importance are *Mucor*, *Rhizopus*, and *Aspergillus* in the order given. In addition to these a considerable number of species of hyphomycetous molds are occasionally found. *Torulæ*, but not true yeasts, are usually common. Bacteria are either spore-bearing soil bacilli or cocci. Of the former, *B. subtilis*, *B. mycoides*, and related forms are ubiquitous. *Sarcina lutea* and *Sarcina aurantiaca* and certain other chromogenic cocci are to be found in almost every plate exposed. Since the air does not have a true flora, the species as well as the number of bacteria present must depend entirely upon the character of the environment.

CHAPTER II

MICROBIAL AIR INFLUENCE IN FERMENTATION, DISEASES, ETC.

Air as a Carrier of Contagion.—There are many popular misconceptions of the influence of air upon health. Experience early taught that exposure to the night air in certain localities or to swamp air during certain seasons was generally followed by disease. Naturally, the air itself was held responsible. We know now that certain fevers, malaria, etc., are caused in every instance by infection with specific microörganisms and that these organisms are not usually carried by the air but by insects, such as the mosquito, in water and food. Nor can the emanations from decaying organic matter or sewer gas itself be held to produce disease directly. Before the establishment of the germ theory of disease, leading sanitarians held that sickness was induced by the gases from the decaying organic matter, by the effluvia from cesspools and by sewer gas. However important the places named may be in harboring disease microörganisms, we have learned that the air itself rarely acts as a carrier. Sewer gas has been shown to be unusually free from bacteria. Hazen says, "After many years of experience and long-continued investigation, there is not the slightest reason to believe that infectious diseases are carried by the air of sewers."

Undoubtedly the air does play some part in the carrying of disease germs. In certain diseases, as the exanthemata (smallpox, measles, etc.), the infecting agent may be present on the dry skin and may be blown about and inhaled. This means, however, is not established. In certain nasal, tracheal, and pulmonary infections, the organisms may be spread through speaking, sneezing, and coughing, for the infectious droplets, as has been seen, remain suspended for a time in the air. Pyogenic cocci are present in the mouth and care must be used in surgical operations that the mouth is so protected that none of these organisms gain entrance to wounds. Rarely, if ever, are intestinal infections, as typhoid or cholera, spread through the air. We may therefore conclude that air is of secondary importance as a carrier of infection.

It may be of importance in a crowded workroom, but even under these conditions it is probable that transmission of infection comes about more frequently through actual contact or through food and drink.

Organisms of the Air and Fermentations.—A uniform inoculation with soil bacteria such as produce the nodules on the roots of legumes is obtained over considerable areas through the action of the wind in blowing dust particles. The bacterial flora of milk is to some extent dependent upon air currents as is also the development of the molds necessary to the proper ripening of cheese, such as the Camembert. Acetic, butyric, and other organisms are likewise distributed in this manner. The organisms responsible for putrefaction and decay, the molding and spoiling of foods are wind-borne.

Freeing Air from Bacteria.—Air is most commonly freed from bacteria by sedimentation, for this is the ultimate fate of most dust particles. We have seen that they gradually subside in a quiet atmosphere. When large quantities of pure air are required, dust and bacteria may be removed by passage through a spray of water or through various types of filters, such as cotton, glass, wool, etc. A familiar example of this type of filtration is the laboratory use of cotton plugs in test-tubes. It is sometimes necessary to resort to fumigation to destroy the organisms of the air when an undesirable species is present.

DIVISION II

MICROBIOLOGY OF WATER AND SEWAGE

CHAPTER I*

MICROORGANISMS IN WATER†

Water is necessary in the life of man. Besides its use as a beverage, for cooking, and all domestic purposes, it is largely used in many manufacturing industries; therefore, the study of its chemical and biological content is one of the most important features of modern hygiene. All natural waters contain microörganisms, which gain entrance from many sources.

Under the influence of the sun, sea water evaporates and forms a water vapor, which we call clouds; and these, driven by the wind over the land, are precipitated as rain and in the form of snow or hail.

Most of this water collects from vast areas into brooks, creeks, rivers, lakes, or in subterranean streams, and finally reaches the sea whence it came.

The water vapor arising from the sea or land contains no organisms; but as soon as the vapor is precipitated microörganisms find their way into it. These come from the air and from the soil. Some of them find in water sufficient nutriment for their life and growth; and, because of their constant presence and evident ability to thrive in water, they are sometimes spoken of as belonging to the "*water flora.*" Others, such as the soil bacteria, are found only at certain seasons, as after rain or during flood-time, and flourish only for a time; while some few, such as intestinal organisms that find their way into water, survive for only a short period.

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* Prepared by F. C. Harrison.

† For specific details regarding methods of analysis and a fuller presentation of the subject, readers may consult any of the following excellent books:

1. Savage, W. G.: The Bacteriological Examination of Water Supplies, London, H. K. Lewis, 1906.
2. Horrocks, W. H.: An Introduction to the Bacteriological Examination of Water, London, J. and H. Churchill, 1901.
3. Prescott and Winslow: Elements of Water Bacteriology, 2d Ed., New York, Wiley & Sons, 1913.

Classes of Bacteria Found in Water

The bacteria found in water are here roughly divided into: (a) natural water bacteria; (*b*) soil bacteria from surface washings; (*c*) intestinal bacteria, usually of sewage origin. But there is no strict dividing line between these three groups; for some organisms belonging to the water flora are found in the soil, and *vice versa.* Water draining from manured land frequently contains intestinal organisms. The division, however, is sufficient for all practical purposes.

Natural Water Bacteria.—The natural water bacteria are generally regarded as harmless to man. These organisms are frequently numerous in river, lake, and all surface waters; certain species predominate at one season, and disappear at another. Some of the best known are mentioned below. Several investigators have grouped the bacteria found in water into classes according to their biochemical properties. Where groups are subsequently referred to, the classification is that used by Jordan and followed by many other workers.

B. fluorescens liquefaciens, Group V, together with some closely allied varieties, is probably more frequently found in water than any other form, and is easily recognized by the green fluorescence and liquefaction it produces in gelatin.

B. fluorescens non-liquefaciens, Group VI, as the name implies, does not liquefy gelatin, but produces characteristic colonies with a fluorescent shimmer, is often very abundant in river waters, and is representative of a group comprising *B. f. longus*, *B. f. tenuis*. *B. f. aureus*, and *B. f. crassus.*

Certain organisms which liquefy gelatin and acidify milk—classed by Jordan in his Group VIII—are quite common at certain seasons. Some of these are soil organisms and are closely related to the proteus group; and some of them are *B. liquefaciens*, *B. punctatus*, *B. circulans.*

Chromogenic bacilli and cocci (*Groups XIII, and XIV*) are often present in water. Of those producing red coloring matter, the well-known *B. prodigiosus* is the type of the group; others are *B. ruber*, *B.*

indicus, *B. rubescens* and *B. rubefaciens*. Several yellow and orange organisms are commonly found, such as *B. aquatilis*, *B. ochraceus*, *B. aurantiacus*, *B. fulvus*, etc.

At certain times, particularly in river and brook waters, organisms producing violet pigment are quite common. *B. violaceus* or *B. janthinus*, as it is sometimes called, is the prevailing type; others are *B. lividus*, *B. amethystinus*, and *B. coeruleus*.

The chromogenic cocci produce either orange or yellow pigment, and as a rule are not numerous in water. *Sarcina lutea* is the most common species.

Non-chromogenic cocci (Group XV) are more frequent. *M. candicans*, *M. nivalis*, *M. aquatilis*, are non-liquefying forms, and *M. coronatus* is the type of those which liquefy gelatin.

SOIL BACTERIA FROM SURFACE WASHINGS.—During times of flood, high water, and after rains, numerous soil organisms are found in natural waters; and occasionally certain species persist for a considerable time. Among the commonest species is *B. mycoides*, with its characteristic rhizoid colony; also *B. subtilis*, *B. megatherium*, and *B. mesentericus vulgatus*, with its allied varieties; likewise *B. m. fuscus* and *B. m. ruber*— all belonging to Jordan's Group VII, and having many characters in common, such as characteristic colonies, followed by liquefaction when growing in gelatin, production of spores, etc.

Cladothrix dichotoma, one of the thread bacteria, easily recognized on gelatin plates by the brown halo that surrounds the colony, is often found in fresh and stagnant water, and in most soils. It seems to flourish wherever there is much organic matter.

These are the soil organisms most often found when beef peptone gelatin is used for isolating purposes; but if other media are used, a different flora appears, and we find nitrifying organisms, yellow chromogens, etc.

INTESTINAL BACTERIA, USUALLY OF SEWAGE ORIGIN.—*Proteus Group*.—There are several groups of sewage organisms found in impure water; some of these are very abundant in crude sewage, but are not found in such relatively large numbers in contaminated water. Jordan's Group III contains the organisms belonging to the large proteus group, the principal species being *B. vulgaris*, *B. zenkeri*, *B. mirabilis*, *B. zopfii*, the sewage proteus of Houston, and *B. cloacæ*. All these are frequently found in impure water, and in sewage. In the latter Houston has found as many as 100,000 per c.c. All these organisms are motile, liquefy gelatin, and produce gas in dextrose and saccharose broth, and little or none in lactose; reduce nitrates, curdle milk, produce indol, and give a fecal, disagreeable odor in broth or other media.

Sewage streptococci.—The streptococci found in sewage are probably similar to those found elsewhere; but their appearance in contaminated water may be regarded as indicative of recent sewage contamination, because the bulk of the evidence available seems to show that they are delicate organisms, which rapidly die outside of the body. While it is easy to ascertain their presence in polluted water, it is almost impossible to enumerate them; and they do not furnish such good evidence of sewage pollution as the colon bacillus. They may be said to furnish valuable confirmatory evidence of sewage contamination.

B. enteritidis sporogenes.—This resistant, spore-bearing organism is usually present in the intestinal tract of man; is found in sewage, milk, and dust; and occurs in foodstuffs, such as wheat, oatmeal, rice, etc. On account of its ubiquity and the resistance of its spores, it cannot be considered a good indicator of excretal pollution.

B. coli.—The presence of this organism in potable water is generally accepted as the best bacterial indicator of sewage pollution. It must be remembered, however, that there are many varieties of this organism, to which certain investigators have given specific names, even when the differences from the type organism have been very slight. It may be well to mention some of these, to avoid confusion in the mind of the reader. The true colon bacillus, *B. coli*, or *B. coli communis*, or *B. coli communis verus*, is a short bacillus with rounded ends, motile, forms no spores and is Gram negative, does not liquefy gelatin, produces acidity and coagulation in litmus milk, gives rise to acid and gas in glucose and lactose media, causes canary-yellow fluorescence in neutral red media, and produces indol when grown in peptone water. The term "*Excretal B. coli*" has been suggested as a convenient designation of an organism which possesses the above characteristics.

A saccharose fermenting variety of *B. coli* has been named *B. communior;* and we have a whole series of organisms which differ more or less in various biochemical reactions, or lack some of their positive reactions. To some of these the name "para-colon" has been given; and the name "para typhoid" has been applied to those which more closely approximate to the cultural peculiarities of the typhoid bacillus.

For practical purposes in the analysis of water, these distinctions are unnecessary.

Bact. lactis aerogenes, a short, thick, capsulated, non-motile bacterium related to *B. coli*, is also an intestinal organism, and must be regarded as an indicator of sewage pollution.

B. typhosus.—Very few instances are recorded in bacteriological literature of the direct isolation of the typhoid bacillus from infected water. The organism is not long-lived, even in pure water (eight or ten days); and when exposed to the action of sewage bacteria, its longevity is greatly diminished (not more than five to six days). A few resistant specimens may remain alive for longer periods of time.

Although the typhoid bacillus has been found so infrequently in water, it is well understood at the present time that the purification of the water supply of a town or city produces a marked decrease in the number of cases and in the mortality from typhoid fever, as the following table shows: (See also Fig. 122.)

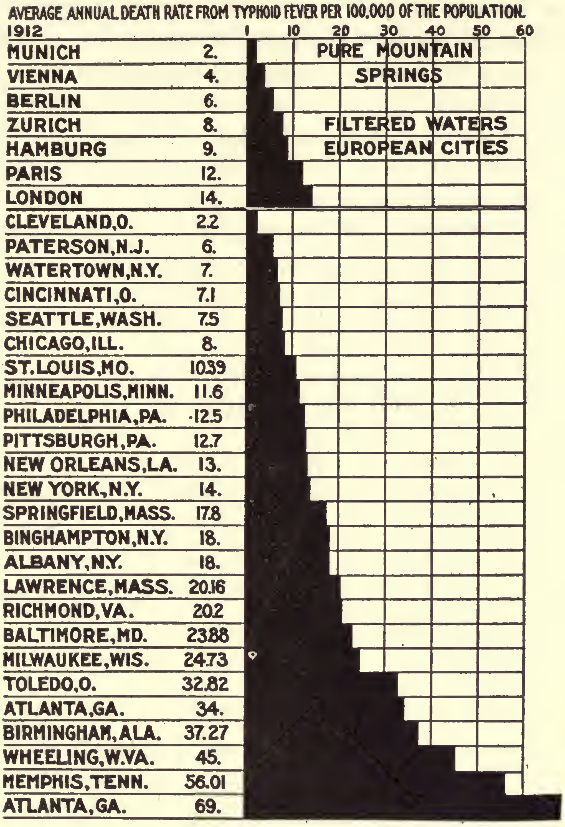
DEATHS FROM TYPHOID FEVER PER 100,000 PER YEAR

Place	Purification by	Date of change	Five years before change	Five years after change	Percentage of reduction
Hamburg...........	Filtration	1892–3	47	7	85
Zürich..............	Filtration	1885	76	10	87
Lawrence, Mass......	Filtration	1893	121	26	79
Albany, N. Y........	Filtration	1899	104	28	73

Not only has such a marked improvement followed the purification of public water supplies in the case of typhoid fever, but it has been shown by statistics that "where one death from typhoid fever has been avoided by the use of better water, a certain number of deaths, probably two or three, from other causes have been avoided."

In the routine examination of water, no particular effort is made to isolate this organism, owing to the difficulty of the task. The tests that the present-day investigator has to satisfy are extremely thorough; and unless the suspected organism conforms to the whole of these necessary tests it cannot be accepted as true *B. typhosus*.

Msp. comma.—The spirillum, or vibrio, of Asiatic cholera is an intestinal organism; and the disease it produces is spread largely by water. Epidemics of cholera are more easily traced to their



An instructive contrast between Altona and Hamburg before the latter filtered its water, having learnt its lesson from a sharp outbreak of cholera.

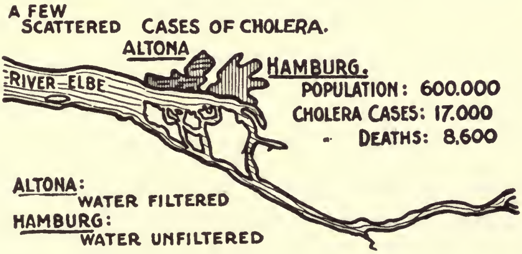

A FEW SCATTERED CASES OF CHOLERA.
ALTONA
RIVER ELBE
HAMBURG.
POPULATION: 600.000
CHOLERA CASES: 17.000
DEATHS: 8.600
ALTONA: WATER FILTERED
HAMBURG: WATER UNFILTERED

FIG. 122.—(*After G. E. Armstrong.*)

source than those of typhoid fever, owing to the "explosive" character of the disease. At the time of the outbreak of cholera in Hamburg, in 1892, the cholera vibrios were frequently isolated from the water of the river Elbe, which was used to furnish the regular supply of the city. The adjoining city of Altona also obtained its water from the same river, after it had received some of the Hamburg sewage; yet it remained practically free from the scourge, owing to the efficiency of sand filters which were used to purify the water (Fig. 122). In times of epidemic, the organism has been isolated from rivers, wells, and reservoirs in India, a country in which the disease is endemic.

THE NUMBER OF BACTERIA IN RAIN, SNOW, HAIL, ETC., AND IN WATER FROM WELLS, UPLAND SURFACE WATERS, RIVERS, AND LAKES

RAIN.—The number of bacteria found in rain depends upon the month of the year and the dryness of the air. When considerable dust is present in the air, the first rain beats it back to the soil; and at such time rain water contains more organisms than usual. Rain falling in densely inhabited cities always contains more microbes than rain falling on open farm land or upland pastures. A few figures will be sufficient to illustrate.

NUMBER OF BACTERIA PER LITER OF RAIN WATER

Figures for Montsouris Park, Paris, France, and the average for two years

Month	Number of organisms per liter	Month	Number of organisms per liter
January	8,000	July	5,600
February	1,320	August	8,300
March	2,920	September	5,770
April	2,140	October	3,220
May	2,440	November	3,250
June	5,600	December	4,330

Yearly average 5,300 per liter per month.

The average for the interior of Paris corresponds with the larger amount of dust in the air, and reaches a total of 19,000 organisms per L. With a yearly rainfall of 609.6 mm. (24 inches), the rain washes down during the year some 5,000,000 organisms to the square yard.

Snow.—The results obtained from snow are similar to those obtained from rain; but as a rule the numbers are larger, a result doubtless due to the larger particles of the snow flakes. One investigator has found from 334 to 463 bacteria per c.c. of snow water. On the summit of high mountains snow is practically sterile, Binot not finding a single organism in 8 c.c. of water from mountain-top snow.

Water issuing from glaciers is of remarkable purity, containing only from three to eight organisms per c.c.; but the numbers are larger as the distance from the glacier increases.

Hail.—Hail stones usually contain large numbers of bacteria, varying from 628 to 21,000 per c.c. of water obtained from the melting hail. Fluorescing bacteria have been found in some samples; and the presence of these microörganisms suggests that surface water is sometimes carried up by storms and congealed. The presence of many molds in hail is due to contamination from the air.

Deep Wells.—Deep well water and spring water contain as a rule but few organisms, usually less than 50 per c.c. on gelatin at 20°, and less than 5 per c.c. on agar plates at blood heat. In a series of tests of water taken direct from forty-three artesian wells, 152.4 M. (500 feet) deep or more, the writer has found an average of 27 per c.c. for the gelatin and 1.5 per c.c. for the agar counts. These tests have extended over a period of several years; and water from deep springs has given similar results.

Shallow Wells.—The bacterial content of shallow wells depends greatly on their location and construction. Even in those well located and constructed, the number varies with the amount of rainfall, and is often large. In polluted wells, very high numbers of organisms are found.

Sedgwick and Prescott found from 190 to 8,640 bacteria per c.c. in unpolluted wells.

In the same class of wells, Savage found from 10 to 100 per c.c. by the blood-heat count, and 100 to 20,000 or more by the gelatin count.

Sixty polluted wells examined by the writer gave an average gelatin count of 740 bacteria per c.c.; and thirty-eight wells which were free of contamination gave an average count of 400 per c.c.

Polluted wells often give counts approximating the higher numbers mentioned above; but, of course, the character of the bacterial flora is quite different.

Upland Surface Waters.—There are few bacteria in upland surface waters draining barren uplands. Cultivation, grazing of animals, and human habitation produce other conditions. In pure waters, 50 to 300 per c.c. by the gelatin and 1 to 10 by the agar count are found.

Rivers.—The greatest variation in the number of bacteria exists in river waters. Many factors, such as sewage contamination, temperature, rain fall, vegetable débris, etc., influence the microbial population. A few figures may be given for illustration.

Bacteriological Examination of Rivers at and below Large Sources of Pollution (Boyce and Co-workers)

Distance	Direction	Munich. River Isar	Cologne. River Rhine
	Above	305	4,786
About 0.6 mile	Below	9,387	
About 2.7 miles	Below	13,503	
About 6.0 miles	Below	8,764	30,432
About 12.0 miles	Below	4,796	12,460
About 15.0 miles	Below	3,602	9,595
About 26.0 miles	Below	......	7,869

In the Chicago drainage canal, Jordan found 1,245,000 bacteria per c.c. at Bridgeport; 650,000 at Lockport, twenty-nine miles below; and 3,660 at Averyville, 159 miles below. Below where the sewage of Peoria enters, the number rises to 758,000 at Wesley City, and decreases to 4,800 at Kampsville, 123 miles from Peoria.

The River Rhône contains an average of 75 bacteria per c.c. above Lyons and 800 below. The Dee, 88 above Braemar and 2,829 per c.c. below. Many more similar results are found in the literature.

Lakes.—The water of lakes is generally much purer than river water. Near the shore, the bacterial content is higher than farther out, showing the contaminating influence of habitation. Thus Lake Geneva contains as many as 150,000 bacteria per c.c. near the shore, and further out only 38 per c.c. Other figures are as follows: Loch Katrine, 74 per c.c., Lake Lucerne, 8 to 51 per c.c., Lake Champlain, 82 per c.c.

Sea Water.—There are few bacteria in sea water remote from the coast; but near the shore and in the neighborhood of seaports there may be large numbers.

Examples: 350 M. from Naples, sea water contained 26,000 bacteria per c.c. At a distance of 3 KM., only 10. Samples taken from depths of 75 to 800 M. at distances from 4 to 15 KM. from shore were found to contain from 6 to 78 bacteria per c.c. in surface water, and from 3 to 260 at various depths below.

CAUSES AFFECTING THE INCREASE AND DECREASE OF THE NUMBER OF BACTERIA IN WATER

There is a number of causes which influence the multiplication or diminution of microörganisms in natural waters; and while it is necessary to discuss each of these causes in detail, it must be remembered that a number of them may be simultaneously influencing the increase or decrease.

TEMPERATURE.—In natural waters, a low temperature probably acts injuriously on parasitic bacteria, reducing their numbers; but the bacterial content of water during the hot summer months is generally not so large as during the cooler seasons. Water collected for examination should be analyzed at once; otherwise, contradictory results as to numbers will be found. Usually, in most waters, there is a reduction in numbers for a few hours, followed by a large increase. Very much polluted waters, however, show a marked decrease of intestinal organisms, if the samples are kept cool.

LIGHT.—Although the germicidal effect of sunlight is well known, yet it has not such powerful effects on the bacteria in water. Much depends, no doubt, on the turbidity and speed of the current, the maximum killing effect being produced in shallow, clear and slow-moving water. It has been found by experiment that the germ-killing power of light extends to a depth of 3 M (about 9.84 feet). As a means of purifying water, direct light produces very little effect.

FOOD SUPPLY.—The amount of organic matter in water directly influences the growth of bacteria. Where a large amount of this is present, the number of microörganisms is also large. Rivers containing considerable organic matter derived from vegetable débris, etc., contain, as a rule, more organisms than rivers in which there is but little of such material. Thus the Ottawa River, which drains a large area of forest lands and is characterized as an upland peaty water carrying a rather high percentage of organic and volatile matter, contains throughout the year a larger number of organisms to the cubic centimeter than the water of the river St. Lawrence, which is much clearer and contains much less organic matter. Sewage water is rich in organic matter, and proportionately rich in bacterial life; and bacterial purification is synchronous with a diminution of organic matter.

Jordan remarks in this connection that "in the causes connected with the insufficiency or unsuitability of the food supply is to be found the main reason for the bacterial self-purification of streams."

OXIDATION.—On the surface of waters, in rapids, falls, and tidal rivers, much oxygen is absorbed, and much impure matter is oxidized. Such oxidation is one of the minor agencies in the purification of water.

VEGETATION AND PROTOZOA.—Low forms of plant and animal life, like certain species of algæ, river plants, and the numerous protozoan forms, bring about a reduction of organic matter in water, and thus reduce the amount of food available for bacteria. There is also the antagonism between these forms and bacteria. The chemical products of the higher forms are considered by some authorities to be injurious to bacterial life; and many bacteria are ingested by predatory protozoa.

DILUTION.—Sewage flowing into a river or lake is at once diluted with quantities of pure water, and the amount of available food material is thus diminished; the space occupied by a definite number of bacteria is increased; and it is easy to see that the greater the dilution, the fewer sewage bacteria will be found. An example will suffice to illustrate. The sewage of the city of Ottawa amounts to about 454 L. (100 gallons) per second; and the gelatin count from it gives an average in round numbers of 3,000,000 bacteria per c.c. The yearly mean discharge of the river is about 1,364,511 L. (300,000 gallons) a second; and thus the sewage becomes diluted 3,000 times.

SEDIMENTATION.—Impurities, suspended matter, and bacteria having weight, naturally gravitate to the bottom; and the subsidence of these matters is spoken of as sedimentation.

Lake water being still, sedimentation in it is more marked than in moving water; and such water contains but few bacteria. In slow-moving rivers the influence of this factor is also quite pronounced; and, according to Jordan, "The influences summed up by the term *sedimentation* are sufficiently powerful to obviate the necessity for summoning another cause to explain the diminution in numbers of bacteria" in sewage polluted rivers. The example already given of the self-purification of the Chicago drainage canal illustrates Jordan's contention.

OTHER CAUSES.—There is a number of other causes, not well known nor of sufficient practical importance for more detailed comment, which may increase or decrease the number of bacteria in water, such as the inhibiting action of microörganisms and their products on one another, the effects of pressure, etc.

A peculiar fact, which has never been satisfactorily explained, is the quick death (in three to five hours) of the cholera vibrio in the waters of the Ganges and Jumna. When one remembers that these rivers are grossly contaminated by sewage, by numerous corpses of natives (often dead of cholera), and by the bathing of thousands of natives, it seems remarkable that the belief of the Hindoos, that the water of these rivers is pure and cannot be defiled, and they can safely drink it and bathe in it, should be confirmed by means of modern bacteriological research. It is also a curious fact that the bactericidal power of Jumna water is lost when it is boiled; and that the cholera vibrio propagates at once, if placed in water taken from wells in the vicinity of the rivers.

INTERPRETATION OF THE BACTERIOLOGICAL ANALYSIS OF WATER

In making any analysis of water, all data, such as the kind of water and the particulars regarding collection, transmission, sampling, rainfall, etc., should be given, as these are a great help in interpreting the results. One analysis is rarely sufficient; examinations should be regularly and systematically made.

QUANTITATIVE STANDARDS.—No absolute guide can be given to determine the potable quality of water from the number of microorganisms in it. It may, however, be safely assumed that high bacterial counts indicate a large amount of organic matter. The number of organisms growing in beef peptone gelatin at 20° to 22°, and termed the "gelatin count," should be given. For deep wells and springs, this should not exceed 50 per c.c.; and for shallow wells and rivers, not over 500 per c.c. After rains or floods, these figures might be exceeded, and would not necessarily indicate dangerous pollution.

The number of organisms which develop on beef peptone agar incubated at blood heat, commonly termed the "agar" or "blood-heat" count, is perhaps more important than the gelatin count, as many water bacteria do not grow at blood heat, whereas sewage and soil organisms grow readily at this temperature. The agar count eliminates the water *flora*, but obscures the sanitary results by reason of the presence of soil bacteria. For deep waters, the agar count should generally not exceed 10 per c.c.; and for surface waters, not over 100 per c.c.

QUALITATIVE STANDARDS.—The isolation and identification of specific disease organisms, such as typhoid and cholera microbes from water, is sufficient to condemn such a sample as unfit for use; but, on account of many technical difficulties, it is practically impossible to make such an examination. Apart from a few special cases, when it may be necessary to attempt the isolation of these pathogenic bacteria, the presence of the colon bacillus (*B. coli*) in small amounts of water, is generally looked upon as significant and indicative of sewage pollution. The technical methods used in this isolation and enumeration are many, and may be found in the works cited; but there is considerable difference of opinion as to the *number* of *B. coli* which should condemn a sample of water. Prescott and Winslow state that if the colon bacillus is in "such abundance as to be isolated in a large proportion of cases from 1 c.c. of water, it is reasonable proof of the presence of serious pollution." Savage suggests that *B. coli* should be absent from 100 c.c. in the case of water from deep wells and springs, and should be absent from 10 c.c. in surface waters, such as rivers used for drinking purposes, shallow wells, and upland surface waters.

The streptococcus examination is next in importance as an indicator of sewage. Streptococci should be absent from the amounts of water mentioned above for *B. coli;* and *B. enteritidis sporogenes* should not be present in 1,000 c.c. of water from deep wells, nor in 100 c.c. from surface waters.

SEDIMENTATION, FILTRATION, AND PURIFICATION OF WATER

As areas become more and more thickly settled and towns and cities increase in population, the problem of obtaining sanitary control over the water supply increases in importance. Very few towns and cities are fortunate to obtain their water supply from an unpolluted area. Consequently expensive installation must be made, in order to purify a suspiciously contaminated water by freeing it from organisms injurious to health. There are several methods of accomplishing such purification; and these will be briefly mentioned.

SEDIMENTATION AND FILTRATION.—This method of purifying water has been used for nearly a hundred years; but the great impetus given to this hygienic measure was due to Koch, who showed in 1893 that the proper filtration of Elbe water saved the town of Altona from an epidemic of cholera which devastated Hamburg as a result of drinking unfiltered water. In this system of purification, the water is first stored in large reservoirs, where the effect of sedimentation and storage reduces considerably the number of bacteria. From the reservoir, the water is filtered through sand, gravel, and pebbles, etc., arranged as shown in Fig. 123. This filtration removes from 97 to 99.5 per cent of the microörganisms.

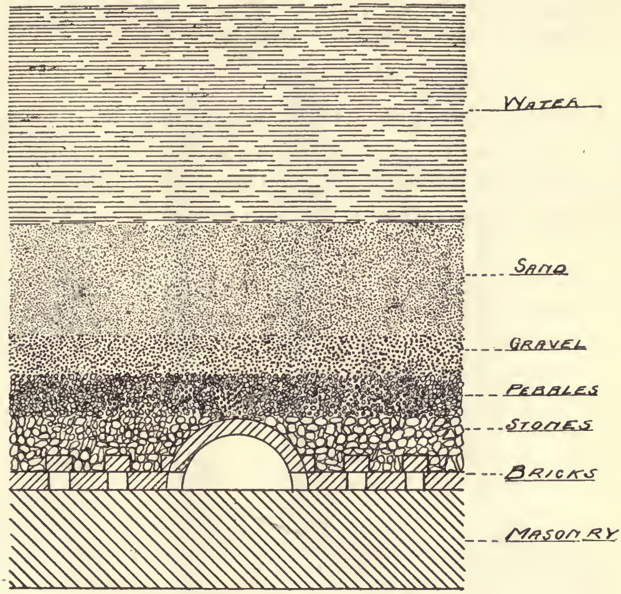


FIG. 123.—Section of a sand filter.

The action of the filter bed is due to the mechanical obstruction of impurities, to oxidation of the organic matter, and to nitrification due

MEAN OF MONTHLY EXAMINATIONS FOR THE YEAR

	Microörganisms per c.c.		
	At source	After storage	After filtration
London, Lambeth Works......	16,138	7,820	75
London, Chelsea Works.......	16,138	1,067	34
Berlin, Lake Müggel..........	1,400	......	60
Paris, Marne..................	79,000	......	630
Paris, Seine..................	186,986	......	400

to the living bacteria in the scum which forms on the top of the layer of sand. Of these, the last is the most important; for until this gelatinous layer forms, the filter does not act properly—in fact, it has little filtering action, as the following figures show:

BACTERIAL CONTENT OF WATER BEFORE AND AFTER CLEANING THE SAND FILTER

Before cleaning, *i.e.*, before removing the scum layer...	42 per c.c.
One day after cleaning..............................	1880
Two days after cleaning.............................	752
Three days after cleaning...........................	208
Four days after cleaning............................	156
Five days after cleaning............................	102
Six days after cleaning.............................	84

Thus provision must be made to permit the scum or film to form before the filtered water is used for domestic purposes.

The rate of filtration must be regulated; for if the water is allowed to exceed a certain rate (101.6 mm. or 4 inches per hour), inefficiency follows.

COAGULATING BASINS AND FILTRATION.—This method of purification consists in adding a coagulant, such as basic sulphate of aluminum, by means of a mechanical device which regulates the quantity, as the water is pumped into the coagulating basins or reservoirs, where it remains for six to twenty-four hours. The aluminum sulphate is decomposed by the lime in the water and forms insoluble aluminum hydrate; and the sulphuric acid combines with the lime. The hydrate of aluminum is precipitated in large flocculent masses, entangling all particles of soil or organic matter; and these, being deposited on the surface of the sand, form the filtering layer. Such filters are very efficient; they remove from 97 to 99.8 per cent of the bacteria from the water.

Porous Filters.—(Fig. 124.) These filters are made either from unglazed porcelain or baked diatomaceous earth; the former are known as Chamberland, and the latter as Berkefeld filters. These filters are usually candle-shaped, require considerable pressure to force water through them, and can be used only when a small supply of water is needed. Water which is forced through these filters is at first sterile; but with repeated use they allow bacteria to pass through the pores and thus the filtering efficiency is impaired and will remain so, until the filters are cleaned and baked to red heat in a muffle-furnace. Unless this is done regularly, no dependence should be placed on these filters, as they only put those who use them off their guard against the danger to which they are exposed.

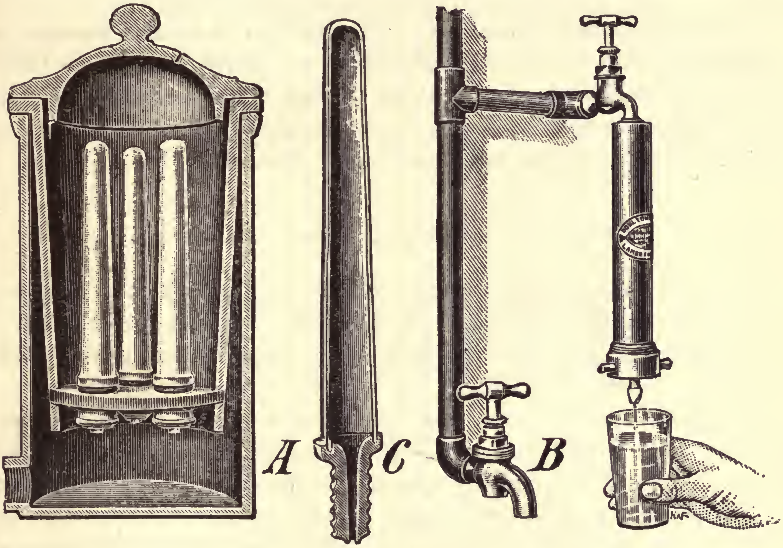


Fig. 124.—Unglazed porcelain filters. Chamberland system; *A*, without pressure; *B*, fitted to main water supply; *C*, section of a porous porcelain filter.

Purification by Ozone.—The antiseptic properties of ozone are well known. It is used in the purification of the water supply of some towns—Nice, Chartres, etc. Ozone used for this purpose is usually obtained by means of the electric current; and a flowing film of water is brought into contact with an upward current of air charged with ozone, which current makes the water almost completely sterile. This method of purification is efficient, but rather expensive.

PURIFICATION BY HEAT.—By bringing water to the boiling point, all harmful bacteria are destroyed; a few spores may resist this treatment, but they are harmless. Boiled water is of a flat, insipid taste, due to the driving out of the contained gases. The taste may be improved by cooling and shaking. The boiling of water is often resorted to as a hygienic measure in times of epidemic, and for the supply of armies in the field.

PURIFICATION BY CHEMICALS.—The addition of a small amount of calcium hypochlorite, or potassium iodide, etc., purifies water; but these methods are seldom used, except for the use of soldiers on campaign. Hypochlorite, however, is now used more commonly in municipal water supplies where they can not be otherwise controlled.

LOCATION AND CONSTRUCTION OF WELLS

Farms in many sections of this country are practically all supplied with surface water collected in shallow wells. Hence farmers should understand the principles involved in the location and construction of wells.

Many farm wells are badly located—too near such sources of contamination as outhouses, cesspools, stables, or barnyards; and those who locate them give too little attention to the slope of the ground, and the nature and slope of the subsoil. There should be at least 22 to 30 M. (75 to 100 feet) between the well and all probable sources of contamination; and this distance is too small, if the soil is very porous, or if the surface and subsoil drainage is toward the well, or if the well is sunk in fissured rock—as it is obvious that there are serious chances of contamination in each of the above circumstances.

In all cases, the surface drainage should be away from the well; and, as far as possible, the subsoil drainage also should be *from* the well.

Sketches 125, 126, and 127 illustrate these points, the upper part of each drawing showing the plan and the lower portion a section through the dotted line marked on the plan. Fig. 125, shows that the surface drainage is from the house, privy, stables, and barnyard toward the well. The section through the line "A" shows the relation of the impervious

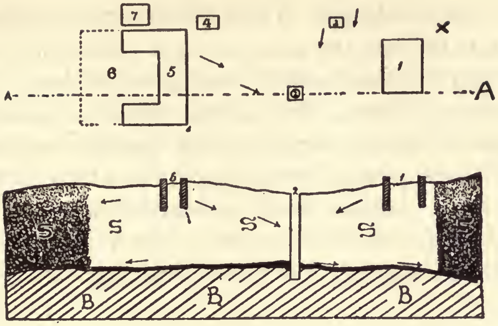

7
4
2
6
5
1
A
A
6
1
2
S
S
S
S
S
S
B
B
B

FIG. 125.

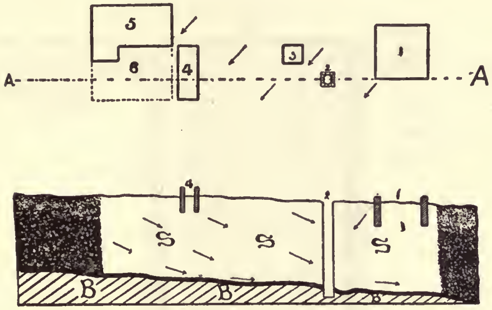

5
6
4
3
2
1
A
A
4
2
1
S
S
S
B
B
B

FIG. 126.

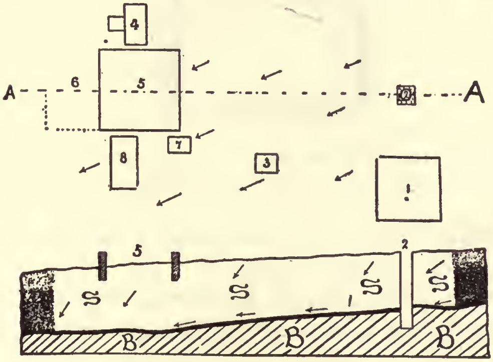

4
6
5
A
A
2
7
8
3
1
5
2
S
S
S
S
B
B
B

FIG. 127.

FIGS. 125, 126, and 127.—In each figure—plan above—section through A B below. S = soil; B = impervious subsoil or strata. 1, House; 2, well; 3, outhouse; 4, piggery; 5, stables; 6, stable yard; 7, hen house; 8, sheep stable. Arrow heads indicate direction of water flow. (Original.)

subsoil "B" to the drainage. Water falling on the surface of the ground would penetrate through the soil to the upper portion of the subsoil, and then move along it in the direction of the greatest slope. In this sketch, the subsoil drainage is away from the well; and in this respect the well is located properly; but, in respect to the surface drainage, improperly located. A better place for the well would be at the letter "X".

In Fig. 126 the surface drainage—including that from the adjacent outhouse at 3, which is too close to the well—is toward the barn, and away from the well; but the subsoil drainage from all the buildings, except the house, is in the direction of the well; and thus contamination of the water supply is liable to occur.

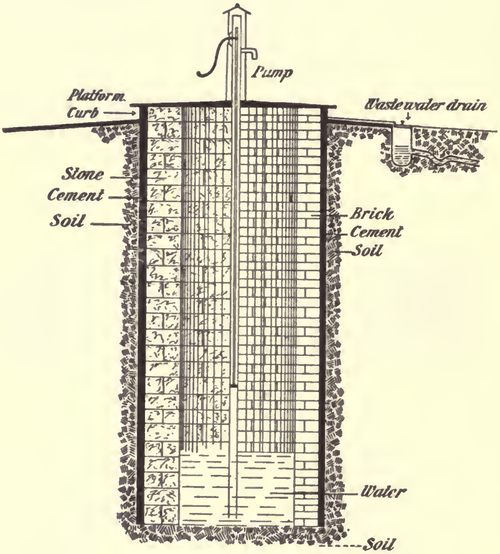


Fig. 128.—Construction of a model well. On the right is brick construction, on the left stone construction, as illustrated. (Original.)

Fig. 127 shows a well properly located as regards both surface and subsoil drainage. Such a well will supply pure water, if it is properly constructed.

Fig. 128 shows the proper construction of a well with brick or stone. Large vitrified drain pipes with cemented joints will answer equally well when there is an abundant supply of water; but in case the supply of water is limited, a large area is needed, and a stone or brick well is necessary.

Reference to the illustrations will show that every endeavor is made to prevent surface water from entering directly into the well. The walls are impervious; and the earth or clay is well rammed against the outer side of the wall. The curb is carried well above the surface of the ground. The waste water is conducted by means of a sloping platform, trap, and drain, away from the well; and the well opening is properly covered. All water entering such a well must percolate through a considerable depth of soil, and undergo purification by means of the aggregations of living bacteria in the soil spaces. Thus the soil around a well fulfils the same function in purifying the surface water as the scum layer that forms on the surface of gravel filters.

CHAPTER II*

MICROBIOLOGY OF SEWAGE

THE BACTERIAL FLORA OF SEWAGE

COMPLEXITY OF FLORA.—Sewage is made up of the miscellaneous and varied wastes of human life and activity, and the bacteria which are found therein are the result of a haphazard and chance admixture of substances of diverse origin and character. The resulting flora is not only of great diversity and variability, but it is with few exceptions non-characteristic. In brief, the medium with which we have to deal has had an origin too indefinite and a history too short to have permitted the establishment of anything approaching a constant or characteristic bacterial flora.

TYPICAL FORMS.—Our interest in this sewage flora is a very practical one, being confined to those organisms which carry on the work of biological purification and to certain pathogens which for obvious reasons require special treatment. We are interested chiefly in what these bacteria do rather than in what they are, and our classification is influenced accordingly. It is based, not upon the species or the genus nor even upon the group or type, that proves so convenient in general bacterial classification, but upon a sort of physiological or functional type, having to do solely with the activities of the organisms in sewage and in its purification. Bacteria performing a common function or producing a common result are members of one type. Individuals may belong to several of our types and there are doubtless a great many that belong to none. These latter simply have no place assigned them as yet in the rôle of sewage purification, because they possess none of the recognized typical functions.

Apparent exception may be taken to these general principles in the case of such organisms as the *B. coli*, sewage streptococci and *B. enteritidis*. These are, to a certain extent, characteristic sewage bacteria. But interest in them as individuals is confined to water bacteriology. If they have any functions in the bacterial changes of sewage, they receive attention as members of a corresponding type, not as individuals. A study of these sewage types, therefore, is a study of the chemical changes induced in the medium by the activities of one or the other group of bacteria.

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* Prepared by Earle B. Phelps.

Types of Sewage Bacteria

According to the general character of the changes which they bring about, sewage bacteria are divided into two large groups, the anaerobic or putrefactive bacteria, and the oxidizing bacteria. In regard to the former, no attention is paid to the fine distinctions that have been made in recent years in connection with the definition of putrefaction. In sewage chemistry putrefaction is that change which takes place naturally in sewage after anaerobic conditions have become established. It involves the reduction of urea, the hydrolysis of protein and of cellulose, the emulsification of fats, the reduction of nitrates and sulphates and possibly of phosphates, and those other changes which are characterized by the withdrawal of oxygen and the hydrolysis of complex molecules. These changes are always noted in sewage under anaerobic conditions and the terms putrefactive and anaerobic change are for the present purposes practically synonymous.

The oxidizing reactions on the other hand might be classed under the general heading of aerobic reactions, except that they constitute only a small portion of the group of reactions which take place normally under aerobic conditions. They are distinguished by the fact that oxygen is added to the molecule, the product always containing more oxygen than the initial substance. Carbon dioxide, water and nitrates are produced, in distinction from methane, hydrogen and ammonia, which characterize the anaerobic reactions. A third type, possessing objective rather than subjective functions, in sewage, is made up of pathogenic and other harmful bacteria. These play no part in our theories of purification and the proof of their presence is generally lacking. For the protection of the public health, it is assumed that they are always present in sewage, and our procedure in sewage disposal is modified throughout in accordance with this assumption.

With these definitions in mind we may proceed to a more detailed study of the bacterial types themselves.

Putrefactive and Anaerobic Bacteria.—Putrefaction or anaerobic fermentation involves the withdrawal of oxygen from one molecule or part of a molecule and the subsequent oxidation of another molecule or part of the same molecule. The energy released in this process is utilized in the vital functions of the organism. This action is neither oxidation nor reduction, or more strictly, they are both taking place simultaneously.

A good example of such a process is the fermentation of urea. The reaction takes place as follows:

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

Carbon is oxidized at the expense of hydrogen, a process which, by itself, is endothermic, that is, requires heat or energy for its maintenance. But the heat of formation of the final product is greater than that of the initial substances and the energy thus liberated becomes available for use by the bacteria. It is in this way that hydrolytic changes of this character play the same rôle in anaerobic reactions that is played by direct oxidation under aerobic conditions.

The Liquefaction of Protein.—One of the most clearly defined and useful types of bacterial activity to be seen in the various sewage disposal processes is that which we term liquefaction. This term is used to denote broadly all those changes by which solid and insoluble organic matter is converted into a soluble condition. The particular process known as protein liquefaction is in the main analogous to gastric digestion. Its one characteristic is the increased solubility of the product. The practical importance of protein liquefaction in sewage disposal is very great and the value of the liquefying bacteria correspondingly high. Nevertheless, aside from our knowledge of analogous processes in digestion and in bacterial putrefaction of albuminous substances, we know almost nothing of the chemistry or the bacteriology of this process. An enormous variety of bacteria are included in this group. The whole process is doubtless the result of a very complicated symbiosis in which various sub-groups of bacteria carry out the initial reaction, from which point other groups carry it through successive stages. Absence of one or another of these groups or of some important species of any group doubtless accounts for the diverse results that are recorded. It is well known that the activities within a septic tank, for example, are seldom twice the same. Gross differences readily apparent to the senses of one versed in such matters certainly exist, and in actual results it is rare to find two tanks doing exactly the same kind of work. Much depends of course upon the chemical character of the sewage itself, but much, that is still unexplained, must eventually be traced to the great diversity of the sewage flora and the complex symbiosis as well as bacterial antagonisms that are involved in the reactions with which we are dealing.

During these reactions proteins and albumins are hydrolyzed by successive stages to albumoses, peptones, amino-acids, amines, and finally to ammonia, carbon dioxide, methane, hydrogen, etc. Simultaneously ammonia, amines, and carbon dioxide are eliminated at each stage as products. The tendency then is toward simple, soluble and gaseous side products, and hence of value in the preliminary resolution of the sewage.

The Fermentation of Cellulose.—The fermentation of cellulose is, next to protein hydrolysis, the most important work of the anaerobic bacteria in sewage treatment. So far as is definitely known this action is usually confined to anaerobic conditions. The fact that fence posts decay first at the surface of the ground, or that wood in general decays more rapidly when it is exposed to only a slight degree of moisture, than when it is immersed in water is only an apparent contradiction. The conditions are aerobic in both cases and aerobic bacteria would not be favored by total immersion but the effect in both instances seems to be due to fungus growths which are more active in the moist wood.

The anaerobic fermentation of cellulose is that which is found typically in marshes and of which the chief products are carbon dioxide and methane or "marsh gas." Nitrogenous food material is also requisite, which accounts for the preserving property of reasonably pure water upon wood.

In the septic tank the solution of cellulose is extremely rapid, and large pieces of cotton cloth or rolls of paper are completely dissolved within a few months. Wood itself is more resistant and withstands the action of the tank for years. This is largely due to the fact that the wood molecule is much more complicated than a simple cellulose molecule, and, among the conifers at least, to the further fact that antiseptic intercellular substances are present.

Chemically considered the action is hydrolytic and can be imitated by prolonged boiling in dilute acids. Pectin substances, starches and finally sugars are produced while butyric and other organic acids, carbon dioxide and methane appear as by-products. Bacteriologically, although it has variously been ascribed to one or another organism, it is probably the result of the activities of many and is possibly not the principal activity of any one of these. In other words, cellulose fermentation is probably a series of side reactions produced during the fermentation of the nitrogenous material rather than a definite reaction upon which the metabolism of any single species depends. This view is strengthened by the general observations that this fermentation is in most cases due directly to enzymes. Viewed in this light it is easy to understand the difficulty that has surrounded the isolation of definite cellulose fermenting organisms. Many have been described, chief of which are *B. butyricus* or *B. amylobacter*, *B. omelianski*, *Sp. rugula.*

The Saponification of Fats.—A third great group of type reactions occurring under anaerobic conditions is the saponification or splitting of fat. Our knowledge of this process is even less definite than of the cellulose fermentations. It is a fact that there does take place in sewage a gradual saponification and emulsification by which the fat loses its identity and mingles with the liquid. This effect is most noticeable in the case of long sewers in which considerable velocities are maintained. In quiescent tanks there is a tendency for the fats to rise to the surface and thus become removed from the influence of this action. Thus in small installations enormously heavy scums form upon the tanks and analysis shows a considerable percentage of fat in this material. In larger systems on the other hand there is less and less evidence of fatty material as such. It is true that there is a deposit upon the walls and tops of such sewers and that small floating objects, like matches, rolling along such a wall will accumulate layers of grease and become eventually the familiar "grease-balls" found in the discharge, but in the main the fatty material has become well disintegrated before the outlet is reached.

In this case also as in that previously discussed it is not believed that the action is a direct result of the activity of any particular organism. The proteolytic changes are accompanied by the freeing of alkaline products, ammonia and amines, which leads to some saponification, and which, in turn, leads to a further emulsification. It has also been demonstrated that bacterial activity is commonly associated with fat saponification and decomposition. Whether specific enzymes are present which assist in this final process or not has never been determined. It is significant to note, however, that where sewages are slightly acid, unaltered fats are much more abundant, even though the acidity is insufficient to prevent vigorous putrefactive changes in the sewage itself.

The Fermentation of Urea.—The fermentation of urea has already been referred to as a typical and simple case of anaerobic decomposition. This reaction has great significance in sewage chemistry since a considerable proportion of the nitrogen of sewage is present initially as urea. Owing to the ease and rapidity with which the reaction takes place, however, no special effort is necessary to bring it about in sewage treatment and it therefore receives brief attention in discussions of the chemistry of sewage. The change to ammonia takes place in the small sewers of the system and it is difficult and generally impossible to detect the presence of urea in sewage. It has even been suggested that certain enzymes present in fecal matter are instrumental in bringing about this change and that the bacteria are only indirectly concerned. It is known, however, that a large number of bacteria of general occurrence have the power to produce this fermentation. Of these the *Bact. ureæ* (Miquel) may be cited as an example.

The Reduction of Sulphates and Nitrates.—The production of sulphuretted hydrogen during the anaerobic decomposition of sewage is commonly noted. This substance may arise in at least two ways. Sulphur, being a constituent of most protein substances, is split off from the molecule in this form during certain types of fermentation. Its formation in these cases is analogous to that of ammonia from protein. The amount so produced is small and is usually neutralized and precipitated by the small amounts of iron and other metals always present in sewage. There is therefore no liberation of the gas itself and it is often said that sulphuretted hydrogen is not formed normally in a septic tank. This conclusion is readily disproved by a simple test of the black residue found at the bottom of such tanks.

A second and more important source of this substance is the sulphate normally present in many sewages. Throughout many parts of the country the water supply contains material quantities of magnesium or calcium sulphate, and upon the sea coast the sewage generally receives more or less salt water.

In these cases the reduction of sulphates to sulphuretted hydrogen is not only of interest bacteriologically but probably exerts an influence upon all the reactions that are going on simultaneously. In fact this example serves excellently to illustrate the great complexity of these anaerobic reactions and the mutual interdependence of each upon all the others. Sulphates, under anaerobic conditions, are a source of oxygen and it is upon oxygen that the course of all these reactions depends. Therefore the presence of sulphates and the possibility of their yielding oxygen may alter the course of the other reactions involved. The products of the protein hydrolysis for example may be profoundly modified by the presence of this additional source of oxygen.

The effect upon the bacteria themselves is also to be considered as a factor quite distinct from the purely chemical effect just described. It has frequently been observed, and in fact would be expected, that the products of anaerobic putrefaction are themselves detrimental to the activity of the organism producing the changes in question. The nature of sulphuretted hydrogen makes it appear quite probable that we are dealing here with a toxic substance that would at least inhibit the activities of certain bacteria and in this way further modify the final result.

The same might be said of almost all the reactions with which we have to deal but this example is cited as a typical one.

It is known in practice that the presence of sulphates in a sewage does lead to a distinct type of anaerobic change which is characterized by the marked blackening of the sewage, the formation of secondary reaction products which precipitate after the removal of the suspended matter of the sewage, the evolution of hydrogen sulphide, an excessive amount of mineral or non-volatile residue in the sludge and the formation of free sulphur upon subsequent aeration of the sewage.

Here again, as in the other types of reaction, it is useless for the present to attempt to ascribe this reaction to any particular species. *Sp. desulphuricans* and *B. sulphureus* have been isolated. A non-liquefying anaerobic bacillus, which reduced sulphates strongly, was isolated from Boston sewage in the writer's laboratory by G. R. Spaulding. Others have been described and there is undoubtedly a large group of organisms capable of bringing about the reaction.

Just as the reduction of nitrates is a function performed by many, perhaps most, anaerobes, so the reduction of sulphates, although a less common function, is still common to many forms. In fact nitrates, sulphates, and phosphates form a series in regard to their reducibility and the effect of their presence upon the reaction as a whole. The phosphates so far as has been recorded are not ordinarily reduced.

Oxidizing Bacteria. *The Production of Nitrate and Nitrite.*—A long series of investigations upon the organisms which oxidize nitrogen began with the Franklands and Winogradski, and has continued to the present day. These have given us much information concerning the habits and functions of the nitrifying organisms. Winogradski's original types were *Nitrosomonas* and *Nitrobacter*, the former oxidizing ammonia to nitrite, the latter completing the oxidation to nitrate. Work upon these organisms constitutes such an important factor in soil bacteriology to-day that more detailed discussion of this nitrifying function is left for another place.

In the earlier days of sewage purification great stress was laid upon the work of these organisms, which was believed to be fundamental. The degree of nitrification was accepted as a measure of the work of the filters and little thought was given to the possibility of oxidizing reactions by other forms. With the development of modern sewage disposal methods, the work of this latter type of bacteria has assumed a more important rôle and the actual work of the nitrifying organism has been found to be of only minor and incidental importance.

Other Oxidizing Reactions.—The great groups of aerobic and facultative bacteria are in general concerned in the oxidation of organic matter. There is nothing specific in this reaction and very little that is characteristic of any special or smaller groups. Under certain special and restricted conditions, typical products are formed by particular species, as in the manufacture of vinegar, and it is possible that a careful study of the complex reactions involved in the oxidation of sewage would show a certain sequence in the order of events and certain definite work being accomplished by definite groups. In other words, symbiosis and specialization doubtless take place to a limited extent. But the fundamental fact remains that the metabolism of the organism demands that organic matter be oxidized for the production of energy. Even though certain food substances may be preferred and certain decompositions be normally produced there is necessarily a great latitude and great adaptability.

For this very reason a study of the individual organism and its action upon specific materials throws no light upon the major problem, which is, given fifty different types of organisms and fifty different fermentable substances, in a mixture, what will be the course of the reaction? Here the preferences, the adaptability and the antagonisms all come into play and while it is impossible to say what has happened or how, it is readily conceived and, in fact, almost apparent, that out of this heterogeneous mixture there will come a homogeneous symbiotic family and an orderly sequence of chemical events, in which metabolic needs and food supply are all delicately adjusted.

PATHOGENIC BACTERIA. *Prevalence and Longevity.*—Owing to its origin and nature, sewage may at any time contain infectious material and for the purposes of the sanitarian it is assumed that at all times the germs of disease are present. Such an assumption is possibly in excess of the actual facts and is only justified because it supplies the only possible hypothesis having an adequate margin of safety. The actual prevalence of pathogenic bacteria obviously depends in the first instance upon the amount of sickness in the contributing community. Furthermore, if, as we are coming to believe, a definite proportion of the population are perpetual carriers of typhoid infection then to just as definite an extent is the bacterial population of the sewage made up of typhoid bacteria from apparently well persons. In addition to these, about five one-hundredths of 1 per cent of the population of American cities are suffering from the disease in acute form. Making due allowance for the extra precautions that are, or should be taken in the care of the dejecta, these persons constitute a definite and fairly constant source of infection.

In the case of the other infectious diseases of the alimentary tract, and, possibly to a less extent in the case of tuberculosis, diphtheria, and many others, these general statements are equally applicable, so that the possibility of the occurrence of infectious material in sewage is not a remote one, but definite and almost quantitatively determinable.

As to the persistence of active pathogenic bacteria in the sewage for any length of time the data are less exact. In the case of typhoid fever, which has been more carefully studied than any other disease, the germs are more persistent in pure water than in impure, but whether this generality can be extended to sewage is debatable. Our best information leads to the belief that any reduction in numbers of typhoid bacteria which may take place within the sewer before discharge is of minor importance and of slight sanitary significance.

Discussion of other pathogens must be in even more general terms. Information is almost wholly lacking and it can only be assumed for purposes of safety that, in so far as organisms of these various types are discharged into the sewer, they will persist to a certain extent in the sewage until it is finally disposed of. If such disposal be by discharge into a stream without purification, then the waters of that stream become polluted with infectious material. Studies recently made by Sedgwick and McNutt have indicated the possibility that many diseases, other than the oft-quoted typhoid fever, may be transmitted in this way.

Life in Septic Tanks and Filters.—With the introduction of the septic tank at Exeter, England, in 1893, the question of the fate of pathogenic bacteria in such a tank was raised. It was even suggested that bacteria, such as the typhoid organism, might multiply in the tank. The question was investigated by Professor Sims Woodhead, who concluded that no organisms capable of setting up morbid changes in animals were discharged from the tank. This negative evidence, however, has little weight in the light of more recent experiments. Pickard introduced an emulsion of typhoid bacteria into this same tank and noted only a gradual decrease. After fourteen days he was able to detect 1 per cent of the initial number. He also reported a removal of 90 per cent of the typhoid organisms introduced into a contact filter. These data must be interpreted in the light of two established facts. The typhoid organism tends to die at a rapid but diminishing rate under any but the most favorable conditions. This results in a rapid decrease at first, with a prolonged survival of a few individuals. This process takes place in sewers, in streams, and, in fact, under most artificial conditions. The second fact of importance is the difficulty of recovering the typhoid organism under experimental conditions like those described.

A thorough study of the bacteriology of sewage and of filter effluents led Houston to conclude that the biological processes at work in a filter or tank were not strongly inimical, if hostile at all, to the vitality of pathogenic germs.

A conservative study of all the evidence bearing upon this important question including the vitality and fate of certain non-pathogenic species, such as *B. coli*, leads to the conclusion that the removal of pathogenic bacteria in purification methods is due to two allied causes, the efficiency of which can be approximately determined. There is first the time element and the known rapid decrease in the numbers of certain bacteria such as *B. typhosus* when placed under conditions that preclude multiplication. The rate of decrease varies but is roughly about 50 per cent in twenty-four hours.

The second factor, acting in reality in conjunction with the first, is the mechanical hindrance that is offered to the free passage of suspended materials through the body of a filter. Even fine sand offers little straining action as such, since the open channels are thousands of times as big as the bacterial cell, but surface tension phenomena tend to make all solid material adhere to the medium and thus its passage is delayed. This action is prominent although of less importance in coarse-grained filters. Actual experiments by the writer have indicated that while the liquid may pass through a trickling filter in half an hour, small suspended particles such as ultramarine and *B. prodigiosus* cells require an average of over twenty-four hours. In this way the actual time of passage is greatly delayed even when coarse broken stone is the filter medium, and the times that are now known to be necessary for the passage are ample in themselves to account for the reductions that have been noted.

It may therefore be stated as a conservative view of the efficiency of purification processes in the removal of pathogenic bacteria, that there are no strongly inimical processes at work in the tanks or filters, and that the rate of decrease is not materially greater than would be observed in the same period of time under the conditions of a running stream.

THE CULTIVATION OF SEWAGE BACTERIA

There are two general methods employed for the cultivation of those bacteria which are of assistance in sewage purification. They may be cultivated in so-called filters of sand or coarser material, or in specially constructed tanks such as the septic or the hydrolytic tank. In the former case the bacterial growth occurs upon the special medium provided, the sand or stone; in the latter, it takes place in the liquid itself and a continuous life history within such a tank is possible only when the rate of flow is sufficiently slow to permit of the inoculation of the incoming stream by the contents of the tank.

FILTERS.—The filtering media most commonly employed are sand or crushed stone or other coarse material. In natural sand beds a

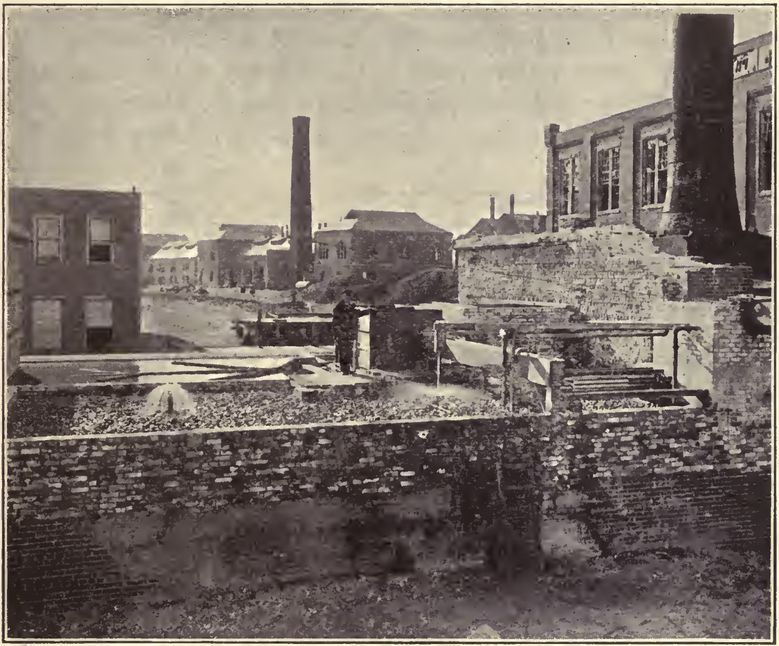

FIG. 129—Sewage Experiment Station, Mass. Inst. Technology. Trickling filter in front, sand filter just behind filter, dosing tank just behind sand filter, and septic tank just behind dosing tank.

brief period of treatment with sewage suffices to produce an active state of "nitrification." By this term is indicated all the complex processes of oxidation one index of which is the formation of nitrates. After such a filter has once become active in this way it will continue, with proper care, to oxidize sewage almost indefinitely. Improper care, such as an overdose of sewage or continued flooding of the surface due to poor drainage, will soon destroy the activity of the filter. The addition of germicidal substances has a similar effect and cold weather somewhat reduces the efficiency. From all this it is apparent that a filter is a biological culture medium upon which the various types of bacteria are growing and carrying out their functions and that such a medium requires careful control and is sensitive to unfavorable changes in environment.

The other filters are similar to this and illustrate the true function of filtration. In the case of the sand filter it might be maintained that filtration or straining was an essential element in the process, but in the case of these coarse-grained media straining action is eliminated. Here there is nothing but a pile of stones, varying from 1 to 3 inches or more in diameter, upon the surface of which the bacteria grow. The sewage trickles slowly over the surfaces, or is held in contact with them temporarily, according as we are dealing with trickling or contact filters. Solids adhere to the stones or settle upon them, and soluble material is "absorbed" by the surface growth and removed from solution. Within these gelatinous growths to which the air also has free access, the processes of oxidation take place and the products, the semi-oxidized organic material, are later "shed" from the stones appearing again in the effluent as humus or stable organic matter.

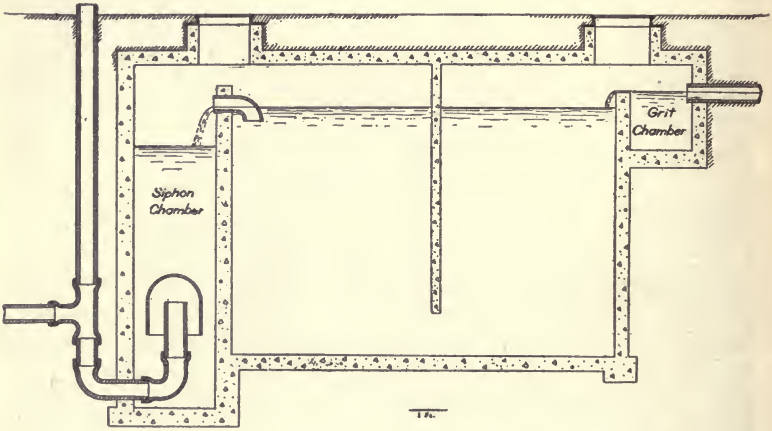

FIG. 130.—Sketch of septic tank. (Original.)

ANAEROBIC TANKS.—The cultivation of bacteria in anaerobic tanks is not quite as simple a matter as that which has just been described.

The sewage is allowed to flow slowly through the tank and after some time, from a few days to a month or more, a normal and constant flora will have become resident there. This flora will soon have become so well established that the incoming sewage laden with a flora of its own mingles with a liquid in which the established flora is so greatly in excess that the former in large measure gives way to the latter. In this way, while the sewage itself moves onward and is gone within a few hours, the flora is constant and persistent. A further aid in preserving this constant flora is the sludge at the bottom, in which the bacteria lodge and multiply and from which they are carried upward by the ever moving eddies and constantly re-inoculate the liquid above (Fig. 130).

THE DESTRUCTION OF SEWAGE BACTERIA

BY BIOLOGICAL PROCESSES.—Reference has already been made to the effect of biological processes of purification upon pathogenic bacteria. What was stated in regard to the pathogens is equally true of the sewage bacteria as a whole. Their destruction is due to time and an environment unfavorable to growth, rather than to any specific cause. Further evidence of these facts may now be given. Bacteria as a whole do pass even the fine-grained filters in large numbers. Careful analyses of their types show them to be a haphazard mixture from the original sewage flora with little or no observable selection. Houston pointed out the relative abundance of the streptococci, supposedly delicate organisms, and found on the whole that the relative abundance of the different kinds of bacteria seemed to be much the same in the effluent as in the crude sewage.

On the whole we may conclude that the biological processes remove bacteria not by any specific antagonistic action but by delaying their passage and permitting the natural decrease that occurs when multiplication is prevented. The more efficient the mechanism of the filter in producing this delay the more complete will be the removal.

BY CHEMICAL PROCESSES.—A much more reliable and economical method for bacterial destruction is now available in chemical disinfection of sewage effluents. The writer's studies at Boston, Baltimore and elsewhere have shown that the application of hypochlorite of calcium in amounts depending upon the character of the effluent, and ranging from one to five parts per million of available chlorine (25 to 125 pounds of bleaching powder per million gallons), will produce a bacterial removal amounting to 98 or 99 per cent. This disinfectant is the most efficient of the known germicides, cost being considered. By this means it is possible to practically eliminate the bacteria, good and bad, from an effluent and it is no longer necessary nor desirable to seek high bacterial removals in the purification process proper. By thus dividing the work of purification into its component parts each part can be carried out at a maximum of efficiency and economy.

DIVISION III *

MICROBIOLOGY OF SOIL

CHAPTER I

MICROORGANISMS AS A FACTOR IN SOIL FERTILITY

INTRODUCTION

Rational views on soil fertility were first presented, in a systematic way, by Justus von Liebig in 1840. In his "Organic Chemistry in its Applications to Agriculture and Physiology" he developed important theories on the circulation of carbon and nitrogen in nature, and on the function of the so-called mineral constituents of plants.

When Liebig's book appeared, many of the leaders and students of agriculture still believed that humus, the partly decomposed residues of plants and animals in the soil, was the direct food of crops. They believed that soils could yield poor or rich harvests in proportion to the amount of humus present in them; they believed, in other words, that plants, like animals, used organic substances as food.

Liebig rendered a great service to agriculture in emphasizing the significance of decay processes. He made it evident that humus as such is of no use to plants, and that it becomes valuable only in so far as it is resolved into the simple compounds carbon dioxide, ammonia, nitric acid and various mineral salts. To be sure, he regarded the decomposition of organic matter as a phenomenon purely chemical, nevertheless he succeeded in showing that decay, putrefaction and fermentation are fundamental facts, connecting links between the world of the living and the world of the dead.

The research of the following decades brought to light the intimate relation existing between microörganisms and the decomposition of

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* Prepared by Jacob G. Lipman with exception of sub-chapter on "*Soil Inoculation*" which has been prepared by S. F. Edwards. The author is indebted to Dr. S. A. Waksman for help in the revision of a portion of the manuscript.

organic matter. In the realm of soil fertility the new discoveries revealed the vastness of the task assigned to soil microörganisms in providing available food for crops. It was shown that under the attack of bacteria and of other microörganisms the various organic débris in the soil is split into relatively small chemical fragments; that the carbon is restored to the air as carbon dioxide; that the nitrogen is changed into ammonia, nitrites and nitrates. It was shown, further, that in this breaking down of organic matter the various cleavage products, and, particularly, carbon dioxide, hasten, to an amazing extent, the weathering of the rock particles and make available thereby the mineral portion of plant food. It was shown, likewise, that apart from accomplishing the *transformation* of unavailable into available plant food, microörganisms are concerned also in the addition of nitrogen compounds to the soil. The evidence gathered slowly by many investigators made it plain, therefore, that microbes are an important factor in the growing of cultivated and uncultivated plants. Hence, the important place assigned to microörganisms in the study of soil fertility problems.

The Soil as a Culture Medium

Arable soils present so wide a range of conditions as to modify, materially, the development and predominance of different species. Variations as to moisture, temperature, aeration, reaction, food supply and biological relations are important, in each case, in determining the survival or disappearance of any particular species. For this reason, the study of soil microörganisms must reckon with the mechanical composition of soils, their ability to retain water and their content of inert and soluble plant food.

Moisture Relations in the Soil

Amount and Distribution of Rainfall.—Precipitation in different regions of the earth's surface varies from practically nothing to more than 1,524 cm. (600 inches) per annum. A portion of this water runs off the surface into the nearest stream, another portion is rapidly changed into vapor and is returned to the atmosphere, and the remainder passes downward, into the soil and becomes the medium in which plant food is dissolved. It is estimated that only about half the total rainfall percolates through the soil. Where the soils are open and nearly level the proportion of percolating water is relatively greater; where the soils are fine-grained and more or less impervious, or the topography broken, the proportion is relatively smaller.

Bacteria and other microörganisms, as well as the higher plants, are directly influenced by the amount of moisture available for their various needs. Hence soil microbial activities are affected not alone by the amount of rainfall, but also by its distribution. It is obvious, for instance, that an annual rainfall of 762 mm. (30 inches) distributed rather uniformly throughout the year would produce different soil-moisture relations than the same amount of precipitation confined to only two or three months. As is pointed out by Abbe, a daily precipitation of 2 mm. (.079 inch) distributed throughout the three summer months would be quickly changed into vapor, and would hardly wet the soil; whereas the total quantity of 180 mm. (7 inches) evenly divided into ten or twelve rains would penetrate the soil to a considerable depth, and would furnish very favorable conditions for microbial development. In a similar manner it is pointed out by Hilgard that Central Montana, and the region in the vicinity of the bay of San Francisco, have each a total precipitation of 610 mm. (24 inches). But while in Montana the rainfall is distributed over the entire year and irrigation becomes necessary, the precipitation near San Francisco is limited to the portion of the year that nearly coincides with the growing season, and crops are enabled to mature without irrigation.

RANGE OF SOIL MOISTURE.—Any given volume of dry soil consists of solid particles separated by empty spaces. The sum of these spaces is known as the "pore-space." It varies from about one-third of the entire volume in coarse sands to more than two-thirds in pipe clay. In peat and muck it may amount to as much as 80 or 90 per cent of the entire volume. Under air-dry conditions each soil grain is surrounded by a very thin film of moisture designated as hygroscopic water. When air-dry soil is moistened the films around the soil particles become thicker and finally cease to be isolated. A continuous liquid membrane, as it were, is stretched from particle to particle, and the surface tension that thus comes into play is capable of lifting large amounts of water to the surface. The continuous film of soil water that can hold its own against the pull of gravity is known as capillary water. Finally, when the liquid films around the soil grains increase in thickness beyond a certain point, the attraction between the molecules in the soil grains and the more distant molecules of water is no longer great enough to overcome the force of gravitation, and the excess of water percolates downward. The water more or less readily moved by gravitation is called hydrostatic water.

For any given conditions of the soils the amount of hydrostatic, capillary and hygroscopic water is directly dependent on the mechanical structure. It is evident that the aggregate surface of the particles in a fine-grained soil is much greater than that in a coarse-grained soil. Actual determinations have shown that the aggregate inner surface of .02832 c.m. (1 cu. ft.) of coarse sand may be but a fraction of an acre; whereas the same quantity of the finest clay may have an inner surface equivalent to 1.2141–1.6188 hectares (3 or 4 acres). These differences are to be expected, since, as is shown by Lyon and Fippin, 1 g. of fine gravel may contain 252 particles; 1 g. of medium sand, 13,500 particles; 1 g. of very fine sand, 1,687,000 particles; 1 g. of silt, 65,100,000 particles, and 1 g. of clay, 45,500,000,000 particles.

Since the soil water is spread as a film over the solid particles and varies in amount with the fineness or coarseness of the soil, and since the quantity of plant food going into solution is determined largely by the amount of water in contact with the soil particles, it follows that clay soils will, under the same conditions, contain more plant food in solution than loam soils and still more than sandy soils. From the standpoint of soil microbiology this is important, for the microörganisms live and multiply in the film water surrounding the soil particles. The concentration of salts in this film water as well as their composition must of necessity affect bacterial activities. In the same way, methods of tillage and cropping affecting the concentration and composition of the film water will modify the chemical changes caused by bacteria and other microörganisms.

Effect of Drought and of Excessive Moisture.—Optimum conditions for plant growth and the development of many important soil bacteria are furnished when about half of the entire pore space is filled with water. In light sandy soils the optimum moisture content may be reached when the wet material contains scarcely more than 8 to 10 per cent of water by weight; while in silt and clay soils the optimum may reach 16 to 20 per cent or even more.

Continued depletion of soil moisture by plant roots and evaporation at the surface causes the film of capillary water to stretch more and more. Finally it becomes very thin, breaks, and ceases to be continuous. The soil then becomes air-dry and contains only hygroscopic water. It is estimated by Lyon and Fippin that, under average conditions of humidity, light sand will contain 0.5 to 1 per cent of hygroscopic moisture; silt loam, 2 to 4 per cent; and clay, 8 to 12 per cent. The amount of water present in air-dry muck or peat may range up to 40 per cent, or even more. According to Hall the film of hygroscopic moisture is about 0.75μ (0.00003 inch) thick. As the soil dries out bacterial activity is suspended and many vegetative cells undoubtedly perish. Nevertheless, it will be seen that the moisture film even in air-dry material is deep enough to allow the bacteria a reasonable degree of protection. This will account for the survival of non-spore-bearing bacteria in dry soil for a long time. Indeed, instances are on record of the isolation of *Azotobacter* and *Nitrosomonas* from soils that had been kept in a dry state in the laboratory for several years. It may be noted, in this connection, that in the process of drying the soluble salts in the soil the moisture may be sufficiently concentrated in the films to cause plasmolysis and the destruction of individual cells.

On the other hand, excessive moisture in the soil is not only directly unfavorable to aerobic species in that it limits their supply of oxygen, but is objectionable because it encourages the formation of reduction products that are toxic to these species. It is apparent, therefore, that favorable conditions for the formation of available plant food by bacteria are created when a certain relation is established between the volumes of moisture and air in the soil. The shifting of this relation in one direction or another is bound to react on species relationships and numbers.

Colloidal Nature of the Soil.—The colloidal condition of the soil imparts to it the ability to absorb substances from their solutions as well as the ability to change them from a flocculated to a deflocculated state. Another important colloidal property of soil is the formation of a colloidal solution in pure water and coagulation by the addition of small quantities of electrolytes. Soluble fertilizers when added to the soil are adsorbed by the latter: otherwise, they could easily be washed out by drainage. The adsorbed substances displace others which may be washed out of the soil.

The addition of ammonium and potassium salts, for example, results in the displacement of the corresponding calcium salts, which can be washed out, and the formation of insoluble nitrogen or potassium compounds which remain in the soil. On adding sodium and magnesium salts to the soil, displacement of some of the insoluble potassium salts may take place and these may become available for plant growth. The interchanges taking place between the salts existing in the soil and those added in the form of fertilizers have an important effect upon soil biological phenomena and plant nutrition. On heating or drying soils, an increase in the amount of soluble food is produced which is probably a result of the change produced in the colloids. It is in this colloidal complex of organic and inorganic compounds, saturated with water and surrounded by the mineral particles that most of the soil biological phenomena take place.

AERATION

MECHANICAL COMPOSITION OF SOILS.—Soil ventilation is an important factor in crop production. It provides for the proper supply of elementary oxygen so essential to decomposition processes in normal soils; for the supply of elementary nitrogen required by nitrogen-fixing species; for the removal of excessive amounts of carbon dioxide; and for the destruction of various toxic substances. The intimate relation existing between soil ventilation and the mechanical composition of the soil material is bound to react on the microbial factors involved. It is well known that the rate of flow of air through soils is inversely proportional to the fineness of the material; in other words, the fine-grained soils, notwithstanding their greater pore space, will not allow air to pass through them as rapidly as coarse-grained soils. King shows, for instance, that 5,000 c.c. of air passed through a column of fine gravel in thirty-seven seconds, whereas in similar columns of medium sand, fine sand, loam and fine clay soil the same amount of air required for its passage 1,178, 44,310, 282,200, and 2,057,000 seconds respectively.

AEROBIC AND ANAEROBIC ACTIVITIES.—The more rapid diffusion of gases from open soils naturally leads to a more frequent renewal of their oxygen supply. In its turn, the latter affects the ratio of aerobes to anaerobes; it follows, therefore, that in clay soils and clay loam soils the activities of aerobic species are retarded to a greater extent than they are in sandy loams or sandy soils. It follows, also, that in fine-grained soils the activities of the aerobes are confined to a shallower soil layer than in coarser grained soils. The reverse is true of anaerobic species. Methods of soil treatment tending to improve soil ventilation react both on the amount of chemical change produced by definite species, as well as the numerical ratio of different species to one another. Among such methods may be included drainage, liming, manuring and tillage.

RATE OF OXIDATION OF CARBON, HYDROGEN AND NITROGEN.—Experiments carried out by Wollny proved conclusively that the production of carbon dioxide in soils is directly affected by the amount of oxygen supplied; that is, by the more or less thorough aeration of the soil. In one of these experiments air containing varying proportions of oxygen and nitrogen was passed through columns of soil. When this air contained 21 per cent of oxygen there were produced for every 1,000 volumes of air 12.51 volumes of carbon dioxide; while with 2 per cent of oxygen in the entering air there were produced only 3.62 volumes of carbon dioxide. Similar observations were made by Schloesing in connection with the formation of carbon dioxide and of nitric acid. Dehérain and many others have recorded the favorable influence of aeration on the rate of nitrate formation, while Lipman and Koch have observed an increased fixation of nitrogen by *Azotobacter*, consequent upon a better supply of oxygen

THE MINERALIZATION OF ORGANIC MATTER.—Conditions that favor the intense activities of decay bacteria lead to a relatively rapid restoration of the phosphorus, sulphur, calcium, magnesium and potassium that had been made fast in plant tissues, to the stock of available plant food in the soil; indeed, in extremely well-aerated soils the decomposition of organic matter and its ultimate mineralization proceed too fast. It often happens that the farmer is unable to maintain a proper supply of humus in these soils because of their openness and is forced to adopt measures that will retard soil aeration. He resorts therefore, to rolling, marling, manuring and green manuring.

On the other hand, heavy, fine-grained soils are not sufficiently well aerated to allow a rapid mineralization of the organic matter. Under extreme conditions the decomposition processes do not keep pace with the process making toward the accumulation of organic matter, and a more or less considerable increase in the amount of the latter takes place. This occurs in low lying meadows, and, more particularly, in bogs and swamps. Hence the farmer attempts to intensify aeration and the resulting mineralization of the humus by more thorough tillage, drainage, liming and manuring.

TEMPERATURE

INFLUENCE OF CLIMATE AND SEASON.—An illustration of the differences that may exist in the soil temperatures of different regions is given by a comparison of the mean temperatures of 1901 recorded at Moscow, Idaho, and New Brunswick, New Jersey. The soil temperatures were taken to a depth of 152 mm. (6 inches).

SOIL TEMPERATURE,* 1901

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Moscow, Idaho...	32.0	30.0	35.0	40.0	52.0	58.0	68.0	72.0	57.0	50.0	40.0	34.0
New Brunswick, N. J.	31.5	28 6	35.3	47.9	57.9	72.1	76.4	73.4	68.5	56.0	41.1	33.4

AIR TEMPERATURE,* 1901

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Moscow, Idaho...	30.0	30.5	38.3	44.0	56.9	55.0	65.5	69.6	50.3	50.5	39.5	39.0
New Brunswick, N. J.	30.8	24.8	39.1	48.3	59.2	70.9	77.4	74.6	67.6	54.6	38.6	32.6

It will be observed that in the months of November to March the soil temperatures in the two places were nearly the same. On the other hand, in April to October the average temperatures at New Brunswick were for soil 14.5° (58°F.) and for air 22.5° (72°F.), respectively; and in July they were 20.0° (68°F.) and 24.5° (76.4°F.) respectively. It will also be observed that there is an unmistakable relation between the corresponding air and soil temperatures.

As a further illustration of the relation of climate to temperature a comparison may be made of the average daily mean temperatures at Bismarck, North Dakota, for the period 1873–1895, and at Key West, Florida, for the period 1872–1895.

DAILY MEAN TEMPERATURES* (AIR)

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec
Bismarck, N. D...	4.5	9.5	22.6	42.1	54.2	63.8	69.5	67.5	57.0	43.8	25.9	14.7
Key West, Fla....	69.7	71.4	72.7	76.1	79.4	82.5	83.9	83.9	82.5	78.5	74.2	70.0

* Recorded in Fahrenheit scale

It is obvious from the figures given here that, because of the important temperature variations of different soil regions, the microbiological activities must be profoundly modified. But apart from the climatic variations already indicated there are seasonal variations in any particular locality that are of great moment for soil microbiological activities. Such differences are demonstrated by the temperatures of 1898 and 1902, taken to a depth of 152 mm. (6 inches), at New Brunswick, N. J.

SOIL TEMPERATURES*

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
New Brunswick, N. J. (1898) ...	33.2	33.1	45.1	48.9	59.1	76.0	79.3	77.8	72.0	60.1	44.6	33.6
New Brunswick, N. J. (1902)....	30.7	28.9	41.3	49.5	60.4	68.0	72.6	70.5	65.9	56.4	48.6	34.1

In this instance, the season of 1898 was not only earlier, but the temperatures of June to September were sufficiently higher to favor more intense bacterial growth and activity.

EARLY AND LATE SOILS.—Under any given climatic conditions the warming up of soils in the spring will depend on their chemical and mechanical composition, color, tillage and topography. Because of the high specific heat of water, fine-grained soils containing a relatively large amount of moisture will warm up more slowly than coarse-grained soils containing a relatively small amount of moisture. The differences in the specific heat of humus, sand, clay and chalk are less important, yet they introduce appreciable variations in the soil temperature according to the proportion of each present. The topography of the soil introduces a factor of some importance for it affects the inclination toward the sun's rays as well as the drainage conditions. Tillage operations are of considerable moment, since they influence the rate of evaporation, that is, the rate at which heat is lost from the soil by the transformation of liquid water into vapor. Finally the color of soils exerts an influence on their temperature in that it affects the absorption and reflection of heat.

Taking all of the factors together, it is found that sandy soils and sandy loams are early soils, because they part readily with their excess of water. Clay soils and clay loams are, on the other hand, late soils; it means, therefore, that in the more open soils microbial activities become intense earlier in the spring. Market gardeners usually attempt to improve matters still further by the use of large quantities of readily fermentable manure that develops enough heat to raise slightly the soil temperature.

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* Recorded in Fahrenheit scale.

PRODUCTION AND ASSIMILATION OF PLANT FOOD.—It was observed by Möller that slight amounts of carbon dioxide may be evolved from frozen soil. Kostychev could detect a considerable production of carbon dioxide at 0° to 5°. In a series of experiments carried out by Wollny the amounts of carbon dioxide produced were as follows:

CO_2 IN 1,000 VOLS. OF AIR

Water in soil	10°	20°	30°	40°	50°
6.79 per cent	2.03	3.22	6.86	14.69	25.17
26.79 per cent.......	18.38	54.22	63.50	80.06	81.52
46.79 per cent.......	35.07	61.49	82.12	91.86	97.48

The increased production of carbon dioxide at the higher temperatures, as shown in the above table, corresponded with the observations that had already been made by Ebermayer, Schloesing and others, that carbon dioxide production in the soil is greater in summer than it is in winter. These facts, taken together with the early observations of Forster on the multiplication of photo-bacteria at 0°, and the more recent observations of numerous investigators on the multiplication of individual species, or of mixtures of species in milk, water, soil, butter, etc., at 0°, or even below that, make it evident that bacterial activities are not entirely suspended at relatively low temperatures. As the latter rises these activities become more intense as gauged by the formation of carbon dioxide.

Coming down to specific groups of soil bacteria, it may be noted that at 12° nitrification is already quite perceptible; that urea bacteria grow slowly at 5°; *Ps. radicicola* at 4°; members of the *B. subtilis* group at 6° to 10°, etc. At 15° the breaking down of organic matter is fairly rapid, and at 25° the optimum is reached for many species. It follows, thus, that the production of plant food—namely, ammonia, nitrates, sulphates, phosphates, etc.—gains rapid headway as the optimum temperatures are approached. The organic matter itself, apart from serving as a source of plant food, furnishes carbon dioxide and various organic acids that help to attack the rock fragments and to render available compounds of phosphorus, potassium, calcium and magnesium. It is likewise evident that in warm countries bacterial activities are not only more intense at any one time, but they continue through a longer period. For this reason, the soils of the South can furnish both relatively and absolutely a greater amount of available plant food than the soils of the North.

The production of plant food is necessarily followed by more vigorous growth of bacteria and of higher plants. More food is, therefore, assimilated and more moisture used up until the very rank growth of the crops hastens the depletion of the soil moisture. In this manner the soil may be dried out sufficiently to retard seriously the growth of soil bacteria and to retard thereby the decompositon of organic matter; under such conditions, moisture, rather than temperature, becomes the controlling factor of growth.

REACTION

RANGE OF SOIL ACIDITY.—Acid soils are very common in humid regions. The older soils of Europe include extensive areas whose lime content has been restored repeatedly by the application of wood ashes, marl, oyster and clam shells, and various grades of burned or crushed limestone. In the United States acidity is becoming prevalent in many of the cultivated soils, as is shown by the investigations of the Rhode Island, Ohio, Illinois, Oregon and Florida experiment stations. These investigations, confirmed by experiments in other states, show that there is a marked removal of lime and of other basic materials from the soil as cultivation and the use of commercial fertilizers become more thorough. Knisley shows, for instance, that 38.75 per cent of the Oregon soils examined were acid, and that 16.25 per cent were strongly acid. Similarly, Blair found that of 189 samples of different Florida soils and subsoils examined, 68.22 per cent of the former and 51.35 per cent of the latter were acid. He also found that virgin soils were less acid than cultivated soils.

CAUSES OF SOIL ACIDITY.—Soil acidity may be due to acids or acid salts, both inorganic and organic. Under ordinary conditions the latter are of much greater importance than the former as a cause of soil acidity. This is demonstrated by the extremely acid conditions of peat and muck soils that are particularly rich in organic acids. In soils left to themselves the formation of basic substances in the breaking down of silicates and other compounds keeps pace with their neutralization by acid and their removal in the drainage water. When soils are placed under cultivation, lime and other bases are removed more rapidly and the inert humic acids are left behind. The loss of bases is intensified by application of acid phosphate, potash salts and ammonium sulphate, commonly used as fertilizers. This accounts for the less extensive acidity in and among virgin soils as compared with cultivated soils. Arid soils lose scarcely any of their basic substances by leaching and are seldom acid. Residual limestone soils may be alkaline, neutral or acid, according to the loss of bases they have suffered by leaching. Low-lying soils, including meadows and swamps may accumulate large amounts of organic acids because of their imperfect aeration.

The more recent investigations of the nature of soil acidity have suggested a physical explanation, namely, that the acidity of the soil is due not to the existence of definite humic and other complex organic acids, but rather to selective adsorption. According to some investigators there is a direct adsorption of the base when a soil is treated with a salt solution. Hence, the behavior of the soil towards neutral salts is not due to the presence of organic matter, but to inorganic compounds, probably hydrated silicates. According to others the development of acidity in the salt solution is due rather to an exchange of bases: aluminum is given up from the soil in amounts approximately equivalent to the base adsorbed.

Through the action of microörganisms in the soil, the organic matter is decomposed with the liberation of weak organic acids (oxalic, citric, CO_2, etc.). By the interaction of these acids in the soil solution and the basic material held adsorbed by the soil, soluble salts are formed which are subsequently removed by leaching: the soil can then adsorb more basic material, giving rise to soil acidity.

Soil Reaction and Hydrogen-ion Concentration.—The different methods for measuring the lime requirements of soils are merely attempts to measure the total soil acidity, but not the intensity of the acidity or the active acidity. The latter can only be determined by measuring the hydrogen-ion concentration of the soil. Pure water dissociates, producing equal concentrations of H ions and OH ions indicating neutrality. The product of the concentrations of these ions (water) is constant, about 1×10^{-14}. If the concentration of H ions is greater than that of the OH ions, the solution is then acid. When the concentration of OH ions is greater than of H ions, the solution is alkaline. Total acidity (or potential acidity, to use the expression of Sharp and Hoagland) or alkalinity may be due to undissolved substances or to soluble compounds only partly hydrolyzed or dissociated.

The hydrogen-ion concentration of the soil can be measured both electrometrically and colorimetrically. The work of Gillespie, Sharp and Hoagland and others has brought out the fact that soils vary greatly in the hydrogen-ion concentration, from a high acidity to a high alkalinity. There is a definite correlation between the hydrogen-ion concentration of soils and the occurrence and activities of microörganisms. Gillespie has shown that potato scab (*Actinomyces scabies*) rarely occurs in soils having a hydrogen-ion exponent lower than 4.8 to 5.2.

Gainey called attention to the fact that *Azotobacter* occurs in soils having a hydrogen-ion exponent greater than 6.0, while the more acid soils are practically free from this important group of nitrogen-fixing organisms. Waksman demonstrated the occurrence of *Azotobacter* in cranberry soils that received an application of lime and gave a decided increase in crop, while the unlimed soil was too acid for the organisms to act in; this limiting reaction for *Azotobacter* corresponded to a hydrogen-ion exponent of about 6.0.

Change of Reaction Produced by Microörganisms in the Medium.—Microörganisms modify the reaction of the medium both by their ability to produce organic and inorganic acids (in the case of sulphur oxidizing bacteria) and also by their utilization of the organic acids as sources of energy.

Effect of Reaction on Numbers and Species.—Some of the important groups of soil bacteria including nitro, azoto and ammonifying species will develop slowly or not at all, when the amount of acid in the medium is increased beyond a certain point. Hence it is realized by progressive farmers that a proper supply of lime is essential for the satisfactory decomposition of organic matter in the soil, and the abundant supply of available nitrogen compounds, as well as of other constituents of plant food to growing crops. The influence of lime on the multiplication of soil bacteria is well illustrated, for instance, by the experiments of Fabricius and von Feilitzen. These investigators found only 138,500 bacteria per g. in newly broken and unlimed peat soils; whereas in similar soils that had been limed and cultivated for several years the numbers averaged about 7,000,000 per g. and reached a maximum of 22,132,000 per g.

Food Supply

Organic Matter.—It may be said truly that a soil devoid of organic matter is practically devoid of bacteria. To the fresh and the partially decomposed organic matter (humus) the soil organisms must look for most of their food and energy. Being largely of plant origin this organic matter contains starches, fats, organic acids, higher alcohols, proteins and amino-compounds. Because of the different relations that these vegetable substances bear to the several species of soil bacteria, a high or low proportion of starch, of cellulose, or protein must necessarily modify both numbers and species relationships. For instance, observations have been made by Coleman and others that small amounts of dextrose favor nitrification, whereas larger quantities retard it; similarly, it has been noted that in the spontaneous decomposition of protein bodies bacteria are prominent and molds absent or relatively few in numbers. But where dextrose is added to the decomposing proteins molds soon appear in large numbers. There may also be cited, in this connection, the observation of Hilgard that humus should contain at least 4 per cent of nitrogen if it is to furnish a sufficient quantity of available nitrogen compounds; otherwise, the soil bacteria seem to be unable to decompose it, so as to meet the needs of the growing plants. Many similar facts could be cited to show that as a culture medium the soil is influenced by green manures, barnyard manure, commercial fertilizers, lime, tillage and any other treatment that will modify the quantity as well as the quality of its organic matter.

The Mineral Portion of the Soil.—The moisture films surrounding the soil grains contain in solution substances derived from these soil grains. A particle of calcium carbonate will be surrounded by a moisture film containing some calcium bicarbonate. In the same way particles of feldspar may give rise to a solution of potassium bicarbonate; particles of apatite to a solution of calcium phosphate; particles of selenite to a solution of calcium sulphate; particles of protein to a solution of ammonia, etc. In view of the fact that these reactions are more or less localized and diffusion slow, there are, undoubtedly, in the soil minute zones where individual species are more prominent than they are in others. For example, Heinze has found it convenient to isolate *Azotobacter* by inoculating suitable culture solutions with particles of calcium carbonate picked out from the soil. Evidently these organisms were present in much greater abundance on these particles than on others of non-calcareous origin. Indeed, he occasionally obtained in this manner *Azotobacter* membranes that constituted almost pure cultures. The more general significance of this relation is apparent when it is remembered that nitro-bacteria are particularly favored by magnesium carbonate; tubercle bacteria by gypsum and calcium carbonate; *Azotobacter* by calcium phosphate and calcium carbonate; photo-bacteria by sodium chloride, etc.

Considerable as must be the local differences in any one soil, they are undoubtedly even more pronounced when different soils are compared. Extreme conditions are met with in certain irrigated soils in which a marked concentration of salts occurs. In so far as crop production is concerned, it is stated by Hilgard that the upper limit is practically reached when the concentration of soluble salts in the irrigation water is about 4.55 g. (70 gr.) per gallon. Nevertheless, in Egypt and the Sahara region irrigation water is occasionally used that contains more than 13 g. (200 gr.) of soluble salts per gallon. Further differences are introduced by the quality of these salts, *e.g.*, the proportion of sodium sulphate, magnesium sulphate, sodium chloride, sodium carbonate, etc. Again, instances are on record, as in the investigations of Headden in Colorado and California, where the concentration of nitrates in the soil water is so great as to kill even relatively resistant plants like alfalfa. It is to be shown by future investigations what the effect of the concentration and composition of such salts may be on the soil bacteria.

In humid soils conditions are less extreme, yet even here the variable concentration and composition of the soil solution are of direct moment for the different microörganisms. Granite soils, for instance, are fairly well supplied with phosphoric acid and abundantly with potash, but when hornblende is lacking they are apt to be deficient in lime. Ill-ventilated clay soils may contain reduction products of iron salts, while green sand, chalk, slate, shale, sandstone and other soils may have their individual peculiarities from the standpoint of a culture medium.

BIOLOGICAL FACTORS

MOLDS.—*Distribution.*—While the study of the lower bacteria in the soil has attracted the attention of many investigators, that of fungi and actinomyces received, until recently, but scant consideration. Fungi occur in all soils, cultivated as well as uncultivated, rich or poor in organic matter, heavy or light in texture. Most of them are obligate saprophytes, although facultative parasites are found in large numbers in the soil, especially where single-cropping or short rotations favor the survival of the particular organisms. The isolation of soil fungi has been accomplished either by the dilution method, where a sample of soil was shaken with water, and only a certain dilution was used for inoculation; and by the direct method, where a clump of soil was inoculated into a sterile medium, and the fungi developing on it were isolated. About 150 different species of fungi have been isolated from different soils, and the data accumulated by investigators in this country and in Europe seem to point to the fact that many of these fungi are universal in their habitat, since the same species are recorded to have been isolated from different soil types and in different localities. Most of the work done refers to the classification of the organisms isolated. The largest group of soil fungi belong to the following genera: *Mucor*, *Zygorrhynchus*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Cephalosporium*, *Monilia*, *Cladosporium*, *Alternaria*, and *Acrostagmus*. Many other genera have been isolated, but to a more limited extent. As to the individual species occurring in the soil, Hagem, having isolated about 30 mucors from the soil, states that certain *Aspergilli* occur in larger numbers than all the mucors taken together. As to quantitative relations, no exact data are available. Some investigators report only several hundred fungi per g. of soil, while others record as many as 1,000,000 per g. of soil; that is the total number of spores and pieces of mycelium that develop on suitable media. As to the numbers and types in relation to depth, Goddard concluded that there does not seem to be an appreciable variation in numbers at the different soil depths. There are very few fungi in the soil below 8 inches and one of the most common forms at these depths is *Zygorrhynchus vuilleminii.* It was formerly thought that soil fungi are abundant only in acid soils, but recent investigations make it appear that also limed and well-cultivated soils have an abundant fungus flora.

The plate count is not an index of the activity of the molds in the soil, but merely indicates the number of spores present. An organism that produces a large number of spores, particularly when these resist drying, will be found by the plate method in much larger numbers than another organism which, though causing a greater degree of chemical change in the soil, produces fewer and less resistant spores. A method was therefore suggested by Waksman which would permit the separation of molds which produce mycelia abundantly and readily from those that develop in soils only under special conditions of moisture and temperature. The method consists of planting a lump of soil into the agar in a Petri dish and observing the development of the mycelia in the soil. It is obvious that the mycelia develop more readily than the spores and grow out into the agar. In this manner we may separate the organisms which actually live in the soil. Moreover, the fact that the same species of molds have been isolated from soils in different parts of the world would tend to indicate that, when conditions become favorable, molds vegetate in the soil, although at other times they may exist there only in the form of spores.

Ammonification.—Müntz and Coudon, and after them Marchal, working with pure cultures, proved conclusively that fungi decompose organic matter and cause an accumulation of ammonia in the soil. Wilson and McLean found that the forms of *Monilia* are the most active ammonifiers among the several groups of organisms studied, while the *Aspergilli* showed the least ammonifying power. More recent work has confirmed the earlier findings and has proved that fungi may play an active part in the decomposition of organic matter, and the accumulation of ammonia.

The molds have been shown to be more rapid ammonifiers than the bacteria and actinomycetes. Species of *Trichoderma* have been found by Waksman and Cook to transform more than 60 per cent of the nitrogen of dried blood and cottonseed meal into ammonia in a period of seven to twelve days. This comparatively rapid ammonia production is readily explained in view of the recent information on the energy requirements of microörganisms. The molds decompose organic matter more readily than do the actinomycetes and many bacteria. They consume a great deal more energy and therefore liberate more nitrogen as a waste product in the form of ammonia.

Nitrogen-fixation.—Experiments on nitrogen-fixation by fungi were carried on by Jodin as early as 1862. He observed a rich fungus growth on nitrogen-free media, supplied with sugar, tartaric acid, or glycerin. Berthelot, Saida, Ternetz, and others also reported fixation of atmospheric nitrogen through the activities of fungi, such as *Aspergillus niger*, *Alternaria tenuis* and several species of *Monilia*, *Penicillium*, *Mucorini* and others. But other investigators, among them Winogradsky, Czapek and Heinze, were unable to confirm these observations. The careful work of Goddard has also given negative results. Duggar and Davis, eliminating all possible errors involved in this study, could not demonstrate any nitrogen fixation for *Aspergillus niger*, *Penicillium digitatum*, *Penicillium expansum*, and other fungi, some of which commonly occur in the soil. Hence, nitrogen fixation by soil fungi is at best of very little importance, since even in the case of positive fixation the amounts are very slight.

Nitrogen Utilization.—The molds assimilate readily available nitrogen compounds in the presence of available carbohydrates. In this respect they may readily compete with higher plants in using up the ammonia and nitrates formed in the soil by bacteria.

Cellulose Decomposition.—The destruction of cellulose in the soil is due to a large extent, to the activities of soil fungi, as has been demonstrated by several investigators. Cellulose decomposition by fungi was first observed in the study of plant diseases. Van Iterson used filter paper for the isolation of fungi, by exposing this medium to the air for twelve hours. Thirty-five species of fungi were isolated thus proving that a large number of cellulose-destroying fungi may be present in the air. Appel found that certain species of *Fusarium* destroyed in fourteen days 80 per cent of the filter paper used. Marshall Ward and others recorded that a number of fungi are economically important as wood-destroyers. Spores of a pure culture of *Penicillium* sown on sterile blocks of spruce wood, germinated and grew normally. Sections of the wood showed that the hyphæ had entered the starch-bearing cells of the medullary rays of the sapwood and consumed the whole of the starch. MacBeth and Scales found that when the medium is slightly alkaline, certain aerobic bacteria will play the principal rôle in the destruction of cellulose. When the medium is acid, molds and higher fungi become the active agents of destruction. They also found that the cellulose-destroying forms multiply with great rapidity in alkaline soils when cellulose in the form of filter paper is added. The power to destroy cellulose is reported for a number of species of *Penicillia*, *Aspergilli*, *Trichodermæ* and other organisms which belong to the common soil forms. Though the fungi may play an important part as cellulose destroyers also in alkaline soils, in acid soils where the activity of bacteria is greatly inhibited, fungi probably play a predominant rôle. This fact led Marshall to conclude in 1893 that fungi take an active part in the mineralization of the organic matter in acid humus soils.

Mycorrhiza.—Apart from the so-called soil fungi, there exists another group known as mycorrhizal fungi. These live symbiotically on the roots of the higher plants. Many roots of forest trees, when examined carefully, show that there is a union between the mycelium of certain fungi, usually belonging to the fleshy fungi, and the root of the plant. This union is called a "*mycorrhiza.*" The fine filaments of the fungus enter the cells of the root. These organisms were thought at first to supply the roots with water and soluble plant food from the soil. The power to fix atmospheric nitrogen has been ascribed to these organisms by several investigators. But aside from these useful so-called endotrophic *Mycorrhizæ*, there are also the ectotrophic *Mycorrhizæ* which probably live only parasitically upon the roots of plants.

Actinomyces.—The study of soil *Actinomyces* is nearly all of very recent origin. Several years ago but two soil *Actinomyces* had been definitely described, viz., *Act. albus* and *Act. chromogenus*. The work of Krainsky, of Conn and of Waksman and Curtis has demonstrated that *Actinomyces* are widely scattered in cultivated soils. The last-named investigators have shown that while the absolute numbers of *Actinomyces* decrease with depth of soil, their relative numbers are materially increased so that if at a depth of 25 mm. (1 inch) there are only 6 to 10 per cent of *Actinomyces* and 82 to 93 per cent of bacteria, at a depth of 750 mm. (30 inches) the *Actinomyces* form 40 to 80 per cent of the total microörganic flora of the soil. The numbers of *Actinomyces* in the surface soil vary greatly with the types of soil and abundance of plant food. In one instance 1,300,000 *Actinomyces* were found in a total of 15,000,000 bacteria per g. of rich meadow soil. The actinomycetes are present in the soil both in the form of spores and vegetative mycelium. The same species have been isolated from North America, Canada, Hawaiian Islands and newly forming soils of Tortugas Island, indicating the universal occurrence of these organisms in the soil. Many species of actinomycetes have been demonstrated to occur in the soil to the extent of millions of cells per gram. As to the activities of *Actinomyces* in the soil, Beyerinck has shown that the *Act. chromogenus* produces an oxidizing substance, quinon ($C_6H_4O_2$) which may play an important part in the oxidation of organic matter in the soil. Munter, Krainsky and Scales have demonstrated that many *Actinomyces* are able to decompose cellulose in the soil, and that in some instances this ability is very marked. Krainsky records that soil *Actinomyces* need very little nitrogen for their life activities, and that they can get it from any available source. If nitrates are present, these are reduced first to nitrites, and then utilized. Waksman and Curtis, working with soil sterilized by steam, did not find any great accumulation of ammonia through the activities of *Actinomyces*, although different species seemed to show marked variation in their power to accumulate ammonia.

ALGÆ.—At times the influence of algæ in changing the character of the soil as a culture medium for bacteria is quite considerable. As chlorophyll-bearing organisms they are enabled to manufacture sugar and starch with the aid of sunlight, and to favor thus the development of *Azotobacter* and of other microörganisms dependent for their energy on the organic matter in the soil. Investigators both in France and in Germany have found that the fixation of nitrogen in sand used for pot culture experiments occurs in the surface layer possessing a growth of algæ. The advocates of bare fallows attribute the greater productivity of fallowed land to the growth of algæ, the accumulation of nitrogen through their influence and to other changes affecting the soil bacteria.

PROTOZOA.—It has been known for a long time that certain species of protozoa are common in soils and that their food consists of bacteria. To what extent protozoa play a part in soil fertility has not yet been fully explained, even though Russell and Hutchinson of the Rothamsted Experiment Station have maintained that these minute animals are extremely important in that they maintain a certain bacterial equilibrium in the soil. Their claim is mainly based on the fact that partially sterilized soils (either by means of heat or antiseptics) soon come to contain enormous numbers of bacteria

It is, therefore, assumed by them that this abnormal increase is made possible by the destruction of the protozoa (which have a lower power of resistance to heat and antiseptics than bacteria) that normally check the increase beyond a certain point. Under the conditions recorded a causal relationship obtains between an increase in numbers of bacteria and the rate of ammonia production, which is considered to be an index of fertility.

This theory has been the basis of considerable investigation, much of which has failed to corroborate the above conclusions. The fact that there is an increase in bacterial numbers and in consequence, enhanced fertility of the soil may not be due to the elimination of protozoa but may rather be ascribed to such effects of the partial sterilization process as (1) increase in available food for bacteria; (2) rendering soil toxins insoluble; (3) destroying bacterio-toxins; (4) acceleration of the biological processes.

It has even been noted in some instances that partial sterilization has been responsible for a decrease rather than increase in the production of ammonia. Such considerations, among others, have been instrumental in stimulating investigation in another branch of soil fertility, namely, soil protozoölogy. There has been difficulty in establishing suitable methods and technic, as for example the development of media favorable for the isolation and culture of soil protozoa, although blood meal solution, hay infusion and soil extract have been used to advantage. The organisms have been counted in the same manner as bacteria, namely, by the dilution method, or by means of a standard platinum loop. An adaptation of the apparatus used in the counting of blood corpuscles has been successfully employed by Kopeloff, Lint and Coleman.

A study of the morphology and life history of soil protozoa reveals the fact that encystment occurs under most conditions which are not immediately favorable, as for example slight variations in moisture content, or food. In point of fact this period of the protozoan life cycle which is analogous to the spore-forming stage of bacteria forms the basis for the question which arises as to the existence of protozoa, in their trophic form, in field soils. Of the well-defined groups of protozoa (page 14), namely, flagellates, ciliates and amœbæ, many types have been described. Among those occurring frequently are: *Colpoda cucullus*, *Boda ovatus*, *Colpidium colpoda*, *Amœbæ terricola*, *Monas*, etc. The requirements for maximum development in the soil for these organisms are: (1) A high degree of moisture, closely approximating saturation; (2) an abundant supply of organic matter; (3) moderate temperature. The thermal death point of active forms has been found by Goodey to be 40° to 50°, and for the cyst forms of the same organisms about 72°. The optimum temperature for most forms is about 22°. Encystment of protozoa occurs within wide limits in an alkaline medium containing up to .18 per cent NaOH, and in the presence of an acidity represented by .09 per cent HCl.

Protozoa are found in many greenhouse soils, due no doubt to the fact that they contain a high degree of moisture and organic matter. However, in dealing with field soils some investigators have failed to isolate active forms of protozoa, whereas others record the presence of large numbers of these organisms. Their distribution appears to parallel that of bacteria, namely, the greatest number of protozoa occurs within the upper 100 mm. (4 inches) of soil, with a decrease down to 300 mm. (12 inches), which represents the lower limit of their activity.

As regards the occurrence of the various groups of soil protozoa, flagellates are found to be dominant over ciliates and amœbæ. G. P. Koch has found that the development of soil protozoa in artificial culture solutions varies (1) with the kind of media employed; (2) the quantity of soil used for inoculation; (3) drying of the soil; (4) different kinds of soil and different soils of the same kind; (5) the temperature of incubation.

While it is generally accepted that protozoa feed upon bacteria, until the relation that obtains between the various types of protozoa and the different species of soil bacteria has been more fully investigated the direct effect of protozoa upon bacteria must remain, to a degree, indeterminate.

Soil sterilization has had a practical application in eliminating various diseases in greenhouses and infested fields. Partial sterilization as employed by Russell and Hutchinson while not so drastic, involves serious changes in the soil, which might be considered in much the same light as the phenomena attending complete sterilization by means of heat and antiseptics. It is an established fact that sterilization is responsible for increased plant growth, and to explain this phenomenon the following theories have been advanced:

1. R. Koch's theory of direct stimulation to plant growth—a physiological effect of the sterilizing agency.

2. Hiltner and Störmer's theory of indirect stimulation—an alteration of the bacteriological equilibrium resulting in a marked development of numbers after decimation.

3. Liebscher's view that soil sterilization may be regarded in the same light as a nitrogenous fertilizer.

4. Russell and Hutchinson's protozoan theory of soil fertility.

5. Pickering and Schreiner's contention that the alteration in chemical composition is largely responsible for increased plant growth.

6. Greig-Smith and others adhering to the bacterio-toxin hypothesis.

HIGHER PLANTS.—Higher plants modify the soil as a culture medium for bacteria in at least three ways. The root-hairs come into contact with the moisture films surrounding the soil grains and not only modify the composition of the film water, by withdrawing a portion of the dissolved matter, but also change its character by secretions from the roots. The changes thus effected must, necessarily, modify the character of the soil and the soil solution as a culture medium. Again, the rapid removal of water from the soil by growing crops causes the film water to become more concentrated in so far, at least, as some salts are concerned. Modifications are, also, introduced thereby in the proportions of oxygen, nitrogen and carbon dioxide in the soil air. Finally, higher plants modify the soil environment for bacteria by their root and stubble residues. For example, residues of leguminous plants, being richer in nitrogen and possessing a narrower carbon-nitrogen ratio than the corresponding residues of non-legumes, will affect the soil somewhat differently than the latter.

BACTERIA.—Occupying, as they do, the leading rôle, bacteria demand a more detailed consideration; in fact, most of the biological discussions of soil are based upon a knowledge of these organisms.

Numbers and Distribution (*Bacteria in Productive and Unproductive Soils*).—The numbers of bacteria in soils well supplied with organic matter usually range from 3,000,000 to 200,000,000 per g., as shown by the agar plate method; the microscopic count will show as high as 900,000,000 per g. of soil. These numbers vary from soil to soil, and from season to season for any particular soil. The numbers of fungi are also variable and may reach a total of 1,000,000 per g., although it still remains to be demonstrated whether the large numbers thus found represent organisms which lead an active life in the soil or only spores of fungi brought in by external agencies. The numbers of

Actinomyces may reach 1,000,000 or more per g. of soil. The fungi almost disappear below 20 to 30 cm., while the actinomyces do not decrease rapidly at depths lower than 30 cm.

Distribution at Different Depths.—Most of the soil bacteria are found in the stratum in which the organic residues are concentrated, that is, in the surface soil. Immediately at the surface the rapid evaporation and the germicidal effect of direct sunshine act as disturbing factors, hence the numbers in the uppermost 25 to 50 mm. (1 to 2 inches) are smaller than in the layer of soil immediately below. Beyond the depth of 20 cm. or 22 cm. (8 or 9 inches) the numbers diminish rapidly. Material from a depth of .6 m. to .9 m. (2 to 3 feet) is nearly sterile in humid regions. Differences occur, however, in keeping with the mechanical composition of the soil. In light, open soils the bacteria are not only carried down to greater depths by the percolating water, but can also multiply there, thanks to better aeration. On the contrary, fine-grained compact soils are more effective in holding back suspended material and do not allow the bacteria to pass downward as readily. Moreover, the less thorough aeration of these soils and the accumulation of toxic reduction products in the subsoil serve as an effective check in the increase of bacteria in the deeper layers.

In irrigated soils of the arid and semi-arid regions bacteria are distributed at much greater depths. Their occurrence 2 m. to 3 m. (8 or 10 feet) below the surface is made possible not only by the better aeration of these soils, but by the penetration of roots to great depths and the accumulation there of considerable amounts of organic matter. The practical significance of distribution appears, among other things, in the use of soil for inoculation purposes; for instance, it is reported by Salström that in making peat soils arable the addition of small amounts of fertile loam increases to a very marked extent their crop-producing power. The efficiency of the inoculating material decreases as it is taken from the deeper soil layers. Similarly, in the use of alfalfa soil for the inoculation of new fields the most efficient material is secured at a depth between 7.62 cm. and 17.78 cm. (3 and 7 inches).

Seasonal Variations of Bacterial Numbers and Activities.—Conn has reported an apparent increase of bacteria in frozen soil. This increase seems to be due to an actual multiplication of the organisms rather than to a mere lifting of the bacteria from lower depths by capillary action. The greatest increase was found to occur during the winter in the slow-growing bacteria and not in those that liquefy gelatin rapidly or in the *Actinomyces*. Conn tries to account for the phenomenon by assuming the existence of two groups of bacteria, winter and summer bacteria. The latter, he thinks, prevents the former from multiplying rapidly in warm weather. Hence, the increase in frozen soils is not to be ascribed directly to the low temperature, but to the depressing effect of the cold upon the summer bacteria. Brown found that the soil bacteria diminish during the fall season with the lowering of the temperature, but, when the soil is frozen, an increase in numbers occurs. He also found frozen soils to possess a much greater ammonifying, denitrifying and nitrogen-fixing power than non-frozen soils. According to him, the lowering of the freezing-point of the capillary water, due in part to the concentration of salts at the time of freezing, may account for the abnormal bacterial activities.

Vass recently pointed out that the apparent increase of bacteria in frozen soils is due to the breaking up of the clumps of cells rather than to growth and multiplication. The bacterial activities are influenced by freezing only in so far as it affects the physical properties of the soil.

Morphological and Physiological Groups (*Morphological Groups*).—Rod-shaped organisms are numerically the most prominent among soil bacteria. They occur at times to the extent of 80 or 90 per cent of the total number. Spherical organisms usually constitute less than 25 per cent of the bacterial flora. Spirilla and sarcinæ are present in slight numbers. Conditions may occur, however, when the proportion of spherical organisms is markedly increased. This happens, particularly, when large quantities of composted manure (rich in spherical organisms) is added to the soil.

Conn has shown that non-spore-forming bacteria (mostly immotile rods) are the most abundant of all soil microörganisms. Next to them in abundance are the various types of *Actinomyces*, referred to elsewhere in this book. Spore-forming bacteria are also quite common, but are apparently of no great importance in normal soil. Among the most prominent soil bacteria are non-spore-forming, slowly liquefying or non-liquefying, short rods; rapidly liquefying, non-spore-forming, short rods with polar flagella represented by *Ps. fluorescens;* spore formers, which seem to come from spores instead of from active organisms. A few micrococci and members of the *B. radicicola* group have been demonstrated.

Among the rod-shaped species *B. mycoides*, *B. subtilis*, *B. mesentericus*, *B. tumescens* and other members of the subtilis group are quite prominent. Members of the amylobacter group are seldom absent. Members of the proteus group and various fluorescens are always present, while *Bact. ærogenes* and allied species are common inhabitants of the soil.

(*Physiological Groups*).—In the decomposition of organic matter in the soil certain important changes in both nitrogenous and non-nitrogenous material are accomplished by definite groups of bacteria. The breaking down of protein substances is accomplished by the formation of ammonia, nitrites and nitrates. These in turn may be transformed back into more complex amino-compounds, peptones, and proteins, or they may be destroyed with the evolution of free nitrogen. Moreover, there are groups of bacteria capable of joining non-nitrogenous organic matter to elementary nitrogen and of producing thus nitrogen compounds. Again, there are groups of bacteria bearing distinct and important relations to the decomposition of cellulose, or the transformation of its cleavage products, methane and hydrogen. There are, likewise, definite groups of bacteria concerned in the transformation of sulphur and its compounds, and of ferrous compounds.

METHODS OF STUDY

METHODS FOR COUNTING BACTERIA.—There are two methods for the quantitative determination of bacteria in the soil: the plate method and the direct count method. By the use of the plate method we can obtain only relative results, since not all soil bacteria are able to grow and develop into colonies even on the most suitable media. The plate method shows cells of bacteria that are able to develop under laboratory conditions but furnishes no direct evidence as to their exact number. Conn therefore suggested the direct count method, already employed successfully in the bacteriological examination of milk. A smear is prepared by spreading 0.1 c.c. of the soil infusion over an area of 1 sq. cm., then stained with Rose Bengal in carbolic acid. The bacteria are colored deep pink or red, while the mineral particles remain uncolored and most of the organic matter is unstained or stained yellow or light pink. The bacteria are then counted by means of an oil-immersion objective and a high power eye-piece. The actual numbers of bacteria detected by the microscope is probably, according to Conn, 5, 10 or over 20 times greater than that indicated by the plate method. The discrepancy is due to the failure of many cells to produce colonies rather than to the occurrence of large clumps that do not break up in the process of plating.

Quantitative Relations.—Since the early work of Koch in 1881 many investigators have determined the number of bacteria in soil samples, by means of the plate method. It is well known, however, that on ordinary gelatin or agar plates kept under aerobic conditions but a fraction of the soil organisms produce visible colonies. The anaerobic species do not appear, nor do aerobic *Azotobacter*, and nitrobacteria, while other common soil organisms form colonies sparingly or not at all. By employing synthetic agar media instead of beef broth gelatin or agar, Lipman and Brown have succeeded in securing the growth of a much larger number of colonies from any given quantity of soil, yet even these larger numbers were incomplete for reasons mentioned above.

H. Fischer recommends a simple medium of agar to which nothing has been added but soil extract (prepared by extracting with a .1 per cent solution of Na_2CO_3) and potassium phosphate. Following the path of Lipman and Brown in reducing the content of organic matter, Temple employed 1 g. of peptone per l. as a culture medium and obtained satisfactory results. Brown has further modified the formula of Lipman and Brown by replacing the .05 g. of peptone with .1 g. of albumin, and obtained results which were somewhat superior. In a comparison of culture media, Conn considers the former media undesirable for quantitative purposes because they contain substances of indefinite chemical composition, and offers an agar medium containing no organic matter except agar, dextrose and sodium asparaginate, and also a soil-extract gelatin which is valuable for qualitative purposes. Other media that have been suggested, after a comparison of all of the above-mentioned media, are the urea-ammonium nitrate agar of R. C. Cook and the tap water gelatine and asparaginate agar of Conn. It is evident, therefore, that the results secured in the counting of soil bacteria have only a relative value. With the same media and methods some information may be secured concerning the influence of fertilization, tillage, liming, etc., on certain of the soil bacteria. But even this information must be properly discounted, since equal numbers do not necessarily mean equal amounts of chemical work accomplished; for example, there is no certainty that 1,000,000 of decay bacteria derived from one soil will accomplish exactly as much decomposition as the same number of similar organisms from another soil. Otherwise stated, individual cells differ in their *physiological efficiency* from other cells of the same species.

Qualitative Reactions.—By modifying the composition of the culture media different physiological groups may be favored in their development. In this manner the silica jelly medium proposed by Winogradski, or the gypsum plates proposed by Omelianski may be employed for making numerical comparisons of nitro-bacteria in different soils. In like manner Beijerinck's mannit agar may be used for the numerical comparison of *Azotobacter*, and other media can be adapted for the quantitative-qualitative determination of urea, denitrifying, methane, and still other physiological groups of microörganisms, modified Czapek's agar and Krainsky's agar can be used for actinomycetes and raisin agar for molds.

There is no doubt that the quantitative-qualitative method just outlined may be made to yield valuable information. Yet it, too, possesses defects already noted in connection with the more strictly quantitative method. Apart from the vast amount of work involved in the preparation of a large number of media and in the counting of colonies on many plates, this method fails to indicate differences in physiological efficiency. Furthermore, the colonies of the specific organisms sought are almost invariably accompanied by those of foreign species not always easily distinguished. With these limitations properly recognized and with further improvement in the constitution of special media the method may be made useful in supplementing data secured by other methods.

Transformation Reactions.—Instead of counting soil bacteria in accordance with colonies produced in general or special media, soil investigators have attempted to measure the bacteriological functions of soils by comparing more or less definite quantities of the latter under known conditions. This method was employed by Wollny and others in studying the factors that affect the formation of carbon dioxide in soils. It was also used by Schloesing and Müntz and their followers in similar studies on nitrate formation. A method somewhat similar in principle but different in its application was proposed by Remy in

1902. He suggested the use of special media for the quantitative estimation of different physiological reactions; thus, making a 1 per cent solution of peptone and inoculating with equivalent quantities of soil, he caused the decomposition of the peptone and the formation of ammonia, and secured comparisons of the ammonifying power of different soils. In a similar manner he used special solutions for comparing quantitatively the transformation accomplished by nitrifying, denitrifying or nitrogen-fixing bacteria.

Remy's method has been extensively tested by Löhnis, Ehrenberg, Lipman and others. It has been shown to possess a serious defect in that it deals with conditions unlike those occurring in the soil itself. For this reason more recent investigations have been carried on in weighed portions of soil rather than in culture solutions inoculated with 10 per cent of soil as is done in Remy's method.

RATE OF OXIDATION OF CARBON.—The rate of decomposition of humus or of other organic matter in the soil may be measured, as was done by Wollny, by determining the amount of carbon dioxide evolved in weighed quantities of material kept under definite conditions. The influence of temperature, moisture, aeration, organic matter, antiseptics, etc., has been determined in this manner. The same method may be used in studying decay, and factors influencing decay, in soils in the field.

More recently Russell and his associates have modified the method in that they have determined the rate of oxidation of carbon not by measuring the carbon dioxide evolved, but by estimating the amount of oxygen absorbed. In either case decay is measured from the carbon standpoint. The method based on this principle should find wide application in future soil fertility investigations.

Potter and Synder measured the amount of carbon dioxide evolved from sterilized soil when inoculated with soil emulsion or with cultures of molds. The latter produced in nearly all cases as much carbon dioxide as the soil suspension and in some cases more. This fact led them to suggest that molds are probably active in normal soils. Gainey pointed out that there is a similarity and agreement between the curves representing carbon dioxide and ammonia formation in soils. The relative content and availability of the carbon and nitrogen sources in the soil influence greatly the relative amounts of carbon dioxide and ammonia produced.

Rate of Oxidation of Nitrogen.—Another method or series of methods for studying decomposition processes in the soil may be based on the determination of nitrogen compounds formed in the breaking down of proteins. Two of the derivatives of protein, namely, ammonia and nitrate, have been used successfully to gauge the decomposition of organic matter in the soil. The recent results secured by Lipman and his associates demonstrate that ammonia formation from dried blood in weighed quantities of soil may serve as a very accurate measure of decay from the nitrogen standpoint. Corresponding determination of nitrates may similarly be employed in tracing protein cleavage and transformation as influenced by the various factors of season, soil and cultivation.

Addition of Nitrogen.—At least one other bacteriological factor in soils should be mentioned here as deserving attention in a systematic study of soil fertility from the nitrogen standpoint. It is known that *Azo-bacteria* are widely distributed in arable soils, and that they are more prominent in some regions than they are in others. The student of soil fertility finds it desirable, therefore, to study azotofication in different soils, and employs (for this purpose) mannit solutions like those proposed by Beyerinck, sand cultures supplied with sugar solutions like those proposed by Fischer, or weighed quantities of soil mixed with sugar as suggested by Koch.

The methods referred to above make possible thus the study of ammonification, nitrification and azotofication under controlled conditions and permit, thereby, the measure of bacteriological factors in soil fertility from the nitrogen standpoint.

Reactions Concerning Calcium, Magnesium, Sulphur and Phosphorus.—In addition to the purely chemical methods available for the study of these constituents, microbiological methods have also been suggested. In some of his still unpublished experiments with *Azotobacter* Lipman employed solutions of mannit in distilled water, provided with small quantities of sterile soils which were to supply the organisms with the essential mineral constituents. In this manner interesting data were secured on the availability of phosphorus compounds in different soils; similarly, Christensen has suggested the use of *Azotobacter* for determining the lime requirements of soils, and Butkevich has experimented with cultures of *Aspergillus niger* in determining the availability of the mineral constituents.

CHAPTER II

THE DECOMPOSITION OF ORGANIC MATTER IN THE SOIL

CARBOHYDRATES

Origin.—The sugars, starches, vegetable gums and allied pectine substances, as well as the cellulose, contained in roots and other crop residues add large quantities of carbohydrates to the soil. The crop residues are augmented still further by green manures and animal manures whenever these are used. A good growth of timothy, for example, may increase the content of organic matter in the surface soil by 250–500 kg. (500 or 1,000 pounds) per acre, and three-quarters of this consists of carbohydrates. In the same manner, a green manure crop, or an application of barnyard manure may add to the land as much as 1,500 pounds, or even more, of carbohydrates per acre. These carbohydrates contain a large proportion of cellulose.

The Decomposition of Cellulose.—Pure cellulose, (page 237), $(C_6H_{10}O_5)_x$ is a rather inert substance, as exemplified by the resistance of cotton and flax fiber to decomposition processes. It is well known, nevertheless, that even cellulose is in the end decomposed and resolved into simple compounds. Plant roots, leaves and stems, as well as the trunks of fallen trees, gradually disintegrate and vanish. Under favorable conditions this may proceed rapidly, as is indicated by the process in septic tanks, or in manure heaps on the one hand, and in open sandy soils on the other. The disappearance of cellulose may be accomplished by (*a*) anaerobic organisms, (*b*) by aerobic organisms, (*c*) by denitrifying bacteria, (*d*) by molds and (*e*) by actinomycetes usually classed as higher bacteria.

The Production of Methane and Hydrogen.—The decomposition of pure cellulose and the formation of methane and hydrogen mixed with other gases was first noted by Popov in 1875. Some years later Tappeiner and also Hoppe-Seyler confirmed Popov's observations that nearly pure cellulose in the form of Swedish filter-paper, or cotton fiber may be fermented by bacteria with the evolution of methane, carbon dioxide and occasionally also of hydrogen. These investigators ascribed the decomposition of cellulose to an organism found by Trécul in decomposing vegetable materials, and named by him *Amylobacter* in 1865, because of the blue color assumed by it when stained with iodine.

Subsequent investigations by Omelianski begun in 1894 and continued through a period of years demonstrated the presence of specific anaerobic organisms in decomposing cellulose. He described two distinct species of long, slender bacilli, assuming the clostridium form when in the spore stage. Morphologically the organisms can hardly be distinguished, but physiologically they show important differences in that one causes the fermentation of cellulose with the production of gases consisting of carbon dioxide and methane, while the gases produced by the other consist of carbon dioxide and hydrogen; hence the one is designated by Omelianski as the methane bacillus and the other the hydrogen bacillus. These organisms do not stain blue with iodine, and do not belong, therefore, to the butyric bacilli designated as *Amylobacter* by earlier investigators. Omelianski's investigations make it appear that the butyric organisms are not capable of fermenting cellulose proper.

In culture solutions containing mineral salts and nitrogen in the form of ammonium compounds the decomposition of filter-paper and other forms of cellulose proceeds with considerable rapidity. Calcium carbonate must be added to neutralize the acids formed, otherwise the fermentation soon comes to a standstill. In one of Omelianski's experiments begun in October, 1895, and ended in November, 1896, 3.3471 g. of cellulose was decomposed by a nearly pure culture of hydrogen bacilli. The products consisted of 2.2402 g. fatty acids, .9722 g. carbon dioxide and .0138 g. of hydrogen, a total of 3.2262 g. which nearly accounts for all of the cellulose destroyed. The fatty acids were made up of butyric and acetic acids with a slight proportion of some higher homologue, probably valerianic acid.

In a similar experiment with an apparently pure culture of the methane bacillus, begun in December, 1900, and ended in April, 1901, fermentation began after an incubation period of about one month, and the entire volume of gas gradually evolved was 552.2 c.c. This mixture consisted of 190.8 c.c. methane and 361.4 c.c. carbon dioxide. The products formed from the 2.0065 g. cellulose consumed included 1.0223 g. fatty acids, .8678 g. carbon dioxide and .1372 g. of methane, or a total of 2.0273 g. The slight difference in weight in favor of the fermentation products falls within the limit of error. These experiments show that about one-half of the fermentation products is gaseous and that the other half consists of acetic and butyric acids.

McBeth has shown that the cellulose-dissolving bacteria are unable to produce gaseous products in cellulose or sugar solutions in which they make a luxuriant growth. The compounds formed, under natural conditions, by the cellulose dissolving bacteria are used by other microorganisms and split into simpler products. The carbon dioxide formed is presumably due in all cases to secondary fermentations. The organic acids noted by early investigators were, for the most part at least, presumably due to secondary fermentations and not to the action of the cellulose-dissolving forms.

The Oxidation of Methane, Hydrogen and Carbon Monoxide.—Aside from cellulose, methane may also be produced from various other carbohydrates, organic acids and proteins. Large amounts of methane are thus contributed to the atmosphere by swamps, manure heaps and low-lying meadows. In a purely chemical way methane may also be set free from volcanoes and mineral springs. The constant additions of methane, ethane, hydrogen and carbon monoxide represent a considerable amount of potential energy. It is important to know, therefore, whether these materials are at all utilized.

That methane may be utilized by bacteria as a source of energy was first shown by Söhngen in 1905. He isolated an organism named by him *B. methanicus* that showed itself capable of growing in inorganic solutions confined over an atmosphere of methane, oxygen and nitrogen. The methane gradually disappeared and there were formed considerable quantities of organic matter. The ability to oxidize methane has been claimed for a number of other organisms by Söhngen and others.

Early observations on the ability of moist soil to cause the oxidation of hydrogen are credited to de Saussure (1838). Many years later (1892) Immendorff called attention to the same fact. It was not, however, until 1905 that the oxidation of hydrogen was shown to be a specific biological process. In that year papers by Söhngen and Kaserer reported experiments wherein inorganic solutions confined under an atmosphere of hydrogen, oxygen and carbon dioxide and inoculated with very small quantities of soil developed a bacterial membrane at the surface. The hydrogen was oxidized and organic matter produced at the expense of the energy set free. The observations just noted have been confirmed by other investigators, by means of mixtures and single species of soil bacteria. Finally it should be added here that *B. oligocarbophilus* previously isolated by Beijerinck and Van Delden is able, according to Kaserer, to oxidize also carbon monoxide.

The Cleavage and Fermentation of Sugars, Starches and Gums.—Sugars (page 233) are a very acceptable source of food and energy for soil bacteria. A culture solution containing suitable mineral salts and sugar ferments readily when inoculated with a small amount of fresh soil. When no combined nitrogen is added, *Azotobacter*, or *B.* (*Clostridium*) *pasteurianus* (or both), may come to the fore. The cleavage products then include alcohols, organic acids and carbon dioxide. With *B.* (*Clostridium*) *pasteurianus* butyric acid is one of the prominent cleavage products. When combined nitrogen is also added to the culture solution other organisms will develop prominently, notably members of the subtilis group, butyric bacteria, aerogenes, etc. In the soil itself the addition of sugar leads to a very marked increase in number and, if acid production is favored, molds may subsequently become prominent. In general it may be said that butyric, propionic, acetic, formic and lactic acid, and ethyl, propyl, butyl and iso-butyl alcohol are common cleavage products.

In the case of starch, pectins and pentosans, similar conditions hold good. Diastatic enzymes seem to be produced by various bacteria as well as by molds and actinomycetes. Members of the subtilis group and *B. fluorescens* seem to be able to transform starch into sugar without difficulty. It needs hardly be added here that the vast quantities of organic acids and of carbon dioxide thus formed must play an important rôle in the breaking down of the mineral constituents in the soil.

Fats and Waxes

Origin and Decomposition.—Plant substances contain varying proportions of fats and waxy materials. In the dry matter of grasses and cereal straw crude fat is usually present to the extent of 1.5 to 2.0 per cent. In hay made from clover and other legumes the proportion of crude fat is rather more than 2 per cent. In cereal grains it may range up to 4 or 5 per cent while in soy beans the content of crude fat is 19 per cent, in germ oil meal 22 per cent and flax seed meal 34 per cent.

Under the influence of enzymes produced by molds, yeasts and bacteria the fatty acids occurring as glycerides are decomposed into glycerin and fatty acids. The extent of fat decomposition, brought about largely by molds in the opinion of some, is shown by numerous experiments with peanut cake, olive press cake, cottonseed meal, almond oil, corn meal, etc. In a number of these experiments *Aspergillus niger* seemed to be particularly efficient in decomposing fats. Analogous decomposition processes may occur in the soil as proved by the experiments of Rubner.

ORGANIC ACIDS

Source.—The cleavage products of proteins include large quantities of amino-acids. The latter are still further transformed and yield a variety of fatty acids. The carbohydrates being present in larger quantities than the proteins are still more important as a source of organic acids. Finally, the fats, gums, and higher alcohols contribute additional quantities of the latter. Among the more simple acids, acetic, propionic, butyric, succinic and lactic are common. The extent of acid production was already indicated in connection with cellulose decomposition by the methane and hydrogen bacilli. Apart from these organisms, organic acids are formed by nearly every important species of soil bacteria; moreover, the tissues of dead plants and animals are not the sole source of organic acids in the soil. According to Stoklasa conditions may occasionally occur in the latter, especially when atmospheric oxygen is excluded, that favor the excretion by plant roots of appreciable quantities of acetic acid.

Aside from the organic acids produced by bacteria, we must also consider the acids produced by molds; among these oxalic and citric acids are most important. Certain members of the *Aspergillus niger* group are able to convert as much as 40 per cent of the sugar in solution into citric acid; the latter is then further oxidized into oxalic acid. In addition to the *Aspergilli*, several *Penicillia*, *Mucors*, *Absidia* and other molds, which have been isolated from the soil, are able to produce citric or oxalic acids, or both. The acid produced in the culture medium is either allowed to accumulate or is further oxidized. *Aspergillus niger* oxidizes sugar first into citric acid, the latter is then oxidized to oxalic acid and finally to carbon dioxide.

Transformation and Accumulation.—Salts of organic acids are suitable as food for a wide range of soil bacteria. *Azotobacter* will readily make use of acetates, propionates and butyrates. A number of denitrifying bacteria will grow vigorously with citrates as the only source of organic nutrients. The fermentation of lactates by butyric bacteria has been known for a long time. The decomposition of malates, succinates, tartrates and valerates may be accomplished by various species, and even simple compounds like formates may yield food and energy to certain soil bacteria, among them *B. methylicus* studied by Loew and his associates. It is evident, therefore, that organic acids are not liable to accumulate in well-ventilated soils. Molds, as well as bacteria, destroy them rapidly, and carbonates, carbon dioxide and water are the final products of the decomposition of non-nitrogenous organic matter.

Notwithstanding the ready decomposition of the more simple organic acids in the soil, it is well known that arable soils are frequently acid. This acidity is largely due to the so-called "humic acids," organic compounds whose composition is not well understood. They are composed, to some extent, of rather complex organic acids or of their acid salts. Continued cultivation seems to favor the accumulation of these acid compounds, partly on account of the diminished supply of lime and of other basic materials in older soils. When these soils are limed the humic acids and acid humates are changed into neutral compounds and are then subject to more rapid decomposition by microorganisms. According to the investigations of Blair the average acid soil in Florida requires 1,500 pounds of lime (CaO) per acre to neutralize the acidity to a depth of 84 mm. (9 inches). This means an acidity equivalent to more than one ton of hydrochloric acid per acre. In peat and muck soils the acidity is equivalent to many times this amount of hydrochloric acid.

PROTEIN BODIES

Amount and Quality.—The protein content of farm crops that leave residues in the soil is variable, but in all cases quite considerable. Dried corn stalks contain 5 per cent of protein, timothy hay 6 per cent, red clover hay 12 per cent or more, alfalfa hay 15 or 16 per cent. Even wheat and rye straw may contain as much as 3 per cent of protein. Cotton-seed meal and other oil cakes, tankage, ground fish, hair and wool waste and dried blood (all used more or less extensively as sources of nitrogen to crops) are made up in a large measure of protein compounds.

Being derived from plant residues, from microörganic, insect and animal remains, and from fertilizers and manures applied, the nitrogen in the soil humus exists, for the most part, in the form of protein compounds. Hilgard reports the following humus and nitrogen content, as based on the analyses of a large number of samples of humid, semi-arid and arid soils.

	(Humus), per cent	(Nitrogen in humus), per cent	(Nitrogen in soil), per cent
Arid uplands	0.91	15.23	0.135
Sub-irrigated arid soils	1.06	8.38	0.099
Humid soils from humid and arid regions (California)	2.45	5.29	0.135
Humid soils from other states	7.01	3.78	0.295

Taking the weight of an acre-foot of dry soil at 2,000,000 kg. (4,000,000 pounds) and multiplying the nitrogen by 6.25 (the factor usually employed for converting nitrogen into protein) we find the protein content of these soils to range from about 11,339 kg. (25,000 pounds) per acre to nearly three times as much. Similarly, the Illinois Experiment Station reports quantities of nitrogen equivalent to 3,175 to 4,989 kg. (7,000 to 11,000 pounds) per acre to a depth of 101.6 cm. (40 inches) in gray silt loams, of the lower Illinoisan glaciation. In the brown silt loams the amount of nitrogen to the same depth is usually more than 4,535 kg. (10,000 pounds) per acre; occasionally it is more than 9,071 kg. (20,000 pounds) per acre. In one instance a black clay loam of the late Wisconsin glaciation is reported to have about 13,154 kg. (29,000 pounds) of nitrogen per acre, to a depth of 101.6 cm. (40 inches). This would be equivalent to more than 81,646 kg. (180,000 pounds) of protein; of course, not all of the nitrogen in the soil exists in the form of protein, some of it occurring as amino-compounds, and a small portion as ammonia and nitrates. Nevertheless, by far the greatest part of it occurs as protein compounds.

The protein compounds of the soil humus must be considered from the standpoint of quality as well as from the standpoint of quantity. It is well known that fresh plant residues are attacked more readily by microörganisms than older plant substances. For this reason soils frequently supplied with fresh organic material supply greater amounts of available food to crops than similar soils whose organic matter consists largely of older residues.

Carbon-nitrogen Ratio.—The decomposition of organic matter is readily influenced by the relative content of nitrogenous and non-nitrogenous compounds. Substances of animal origin yield relatively and absolutely more available nitrogen in a given length of time than substances of plant origin. The difference noted is due largely to the greater proportion of protein in the animal materials; in other words, to the narrower carbon-nitrogen ratio. On this basis Hilgard attempts to explain the adequacy of the small proportion of humus in arid and semi-arid soils. Because of the narrower carbon-nitrogen ratio the humus compounds in these soils are decomposed with greater rapidity and yield a sufficient amount of ammonia and nitrate to supply the needs of the crop.

But when plant substances alone are considered the statement just made requires qualification. It is true that cotton-seed meal or linseed meal, having a narrower carbon-nitrogen ratio, will decay more readily than corn-meal or wheat flour. It is also true that any given plant substance, as it undergoes decay, will lose in proportion more carbon than nitrogen. Older humus has, therefore, a narrower carbon-nitrogen ratio than humus of recent origin. The former is more resistant to decay, however, than new humus. In a concrete way, on the other hand, it may be stated that fresh vegetable material of a narrow carbon-nitrogen ratio will decay more rapidly than fresh vegetable material of a wide carbon-nitrogen ratio. The reverse, nevertheless, is true of vegetable materials in advanced stages of decay. Under any given climatic conditions and in any given soil type, the carbon-nitrogen ratio may give important indications only as to the availability of the humus nitrogen. Lawes and Gilbert, as quoted by Hall, found the following carbon-nitrogen ratio in the organic matter of different soils:

Cereal roots and stubble	43.0
Leguminous stubble	23.0
Dung	18.0
Very old grass land	13.7
Manitoba prairie soils	13.0
Pasture recently laid down	11.7
Arable soil	10.1
Clay subsoil	6.0

Hall concludes, therefore, that humus with a wide carbon-nitrogen ratio is more valuable than humus with a narrow carbon-nitrogen ratio, since the latter will be attacked more easily by the soil bacteria. Brown and Allison indicate that there might be a possibility of applying materials of a wide carbon-nitrogen ratio to supply the deficiencies of organic matter on the basis that the former may have the same or better effect on bacterial activities such as azofication, or non-symbiotic nitrogen fixation.

THE TRANSFORMATION OF NITROGEN COMPOUNDS

AMMONIFICATION. *Experimental Study.*—By ammonification is meant the production of ammonia by bacteria out of protein substances or their cleavage products. That ammonia production in the soil is a biological process was first demonstrated by Müntz and Coudon in 1893. These investigators showed that no ammonia is formed in sterile soils. They also showed that ammonia may be produced out of nitrogenous organic matter by molds as well as by bacteria. Marchal not only confirmed these observations, but proved that various microorganisms differ markedly in their ability to produce ammonia. Of the several species of bacteria tested by him, *B. mycoides* (one of the common soil bacteria) proved itself particularly efficient in the breaking down of nitrogenous materials and the production of ammonia.

Since the publication of these experiments a large number of investigators, both in Europe and America, have studied ammonia production in culture solutions as well as in the soil itself. It has been shown that under favorable conditions the breaking down of protein compounds and the formation of ammonia may be very rapid; for instance, in some experiments carried out by Lipman and his associates the following proportions of nitrogen were transformed into ammonia in the course of six days:

Dried blood	16.74 per cent
Concentrated tankage	56.66 per cent
Ground fish	47.16 per cent
Cotton-seed meal	4.95 per cent
Bone meal	16.65 per cent
Cow manure, solid and liquid excreta	32.60 per cent
Cow manure, solid excreta	5.39 per cent

The experiments were carried out in equal quantities of soil and with equivalent quantities of nitrogen in the different substances. It will be observed that more than 56 per cent of the nitrogen in the concentrated tankage was transformed into ammonia, whereas under the same conditions cotton-seed meal yielded less than 5 per cent.

Mechanism of Ammonia Production.—The relatively large protein molecules are readily broken into larger or smaller fragments. This may be accomplished by purely chemical means, as, for instance, by boiling with acids or alkalies, or by biological activities. Among the first cleavage products albumoses and peptones are quite prominent. These in turn undergo further cleavage and the various amino-acids and their derivatives, as well as ammonia, make their appearance. In so far as the different species of bacteria are concerned, the hydrolysis of proteins seems to depend, to a marked extent, on the ability to secrete proteolytic enzymes. With the aid of such enzymes the proteins are more readily hydrolyzed and further changed into amino- and hydroxy-acids, ammonia and carbon dioxide.

Influence of Soil and Climatic Conditions.—Ammonia production in the soil is affected by (*a*) its mechanical and chemical composition; by (*b*) the amount and distribution of rainfall; by (*c*) the prevailing temperatures; by (*d*) fertilizer treatment; and by (*e*) methods of tillage and cropping. The mechanical composition of the soil influences the proportion of aerobic and anaerobic species, while the chemical composition, particularly that of the humus, influences the rate of multiplication and the character of the chemical transformation accomplished. It is well known, for example, that additions of fresh organic matter intensify the rate of decomposition of the soil humus, and, likewise, ammonia production as has been already demonstrated by Breal. In a more general way it was proved by Lipman and his associates that, with a constant bacterial factor, ammonia production in soils varies with the chemical and mechanical composition of the latter. In some of these experiments 100 g. portions of different soils were each mixed with 5 g. of dried blood, sterilized in the autoclave, cooled and inoculated with equal quantities of infusion from fresh soil. The following amounts of ammonia nitrogen were produced in six days:

Soil	Ammonia nitrogen found
A.	31.62 mg.
B.	68.29 mg.
C.	117.06 mg.
D.	107.16 mg.
E.	156.47 mg.

With all other factors constant, chemical and mechanical differences in the soil used were responsible for striking variations in ammonia production, as indicated by the figures given above.

The influence of temperature and moisture conditions is fully as important as that of the chemical and mechanical composition of the soil. The following data secured by Lipman may be cited in this connection as showing the effect of moisture:

One-hundred-gram quantities of air-dried soil were each mixed with 3 g. of dried blood and varying amounts of water added. The ammonia formed was distilled off and determined at the end of eight days. The amounts of ammonia nitrogen found were as follows:

Water added	Ammonia nitrogen found
0 c.c.	4.13 mg.
1 c.c.	4.13 mg.
3 c.c.	5.40 mg.
5 c.c.	10.64 mg.
7 c.c.	26.37 mg.
10 c.c.	49.57 mg.
12 c.c.	70.71 mg.
15 c.c.	93.90 mg.

It appears, therefore, that ammonia production in soils rises or falls as the rainfall or irrigation is increased or decreased, or as the soil water is more or less thoroughly conserved by proper methods of tillage. In the same way, seasons of high temperature favor ammonification while seasons of low temperatures discourage it. This point is well illustrated by the observations of Marchal that at 0° to 5° only traces of ammonia were formed in his culture solutions; that at 20° ammonia production was quite marked, and that at 30° the maximum was reached. Moreover, apart from the seasonal variations in any one locality, there is a wide range in ammonia production, as we pass from the torrid to the temperate and from the latter to the frigid zones.

Species and Numbers.—Ammonia production is a function common to most soil bacteria. In the earlier experiments of Marchal, seventeen out of the thirty-one species tested were found capable of producing ammonia. Prominent among these ammonifiers were *B. mycoides*, *B.* (*Proteus*) *vulgaris*, *B. mesentericus vulgatus*, *B. janthinus*, and *B. subtilis*. Of a considerable number of soil bacteria tested by Chester all but one were observed to produce ammonia. In Gage's experiments with sewage bacteria, seventeen out of twenty species tested proved to be ammonifiers. Similarly, a number of species tested by the writer, among them *B. coli, B. choleræ suis, B.* (*Proteus*) *vulgaris, B. subtilis, B. megatherium*, etc., all produced ammonia in meat infusions. A mass of additional data, accumulated by different investigators, furnishes further proof that ammonia production is a common function of soil bacteria.

The more prominent ammonifiers, including members of the *B. subtilis* group and certain streptothrices, are numerically important in all arable soils. Their numbers are affected, however, by the amount and composition of the soil humus. It has been found, for instance, that additions of straw and of strawy manure increase markedly the numbers of *B. subtilis* and of other members of the group. An increase in the numbers of certain ammonifiers is caused also by additions of lime or of green manure. For example, in experiments carried out by Lipman and his associates portions of fertile soil inoculated with *B. mycoides* were found to contain, a month later, 2,000,000 of bacteria per g. of soil. In similar soil portions that had also received additions of grass the number was twice as great.

More recent investigations (Temple, Waksman) have shown that ammonification tests are of little value in determining the nature of the microbial soil flora, since the rate of ammonia production is largely controlled by the soil medium. If the soil is suitable, there will usually be found enough microörganisms capable of changing the protein nitrogen into ammonia. Temple has suggested the use of ammonification as a test for soil fitness.

Ammonification should be studied not only in the light of decomposition proteins and protein derivatives in the soil, but also from the point of view of energy sources in the soil. Microörganisms can use both carbohydrates and proteins as sources of energy. There is a great deal more of the carbon compounds oxidized to supply the required energy than there is nitrogen consumed in the normal metabolism of the microbe. The addition to any soil of definite amounts of protein with varying amounts of available carbohydrates will lead to the following results: ammonia will be accumulated in the soil to which the protein alone has been added, the amounts of ammonia increasing with the period of incubation up to a certain point; where only small quantities of carbohydrates have been added there will be at first no ammonia produced, but soon the ammonia will begin to accumulate, so that the actual quantities of ammonia may become in a few days even greater than in the soil where no carbohydrates have been added; in soils to which, aside from the protein, large amounts of available carbohydrates have been added, no ammonia or only traces of it will be found.

Ammonia is produced by microörganisms chiefly in the deannization of the amino acids; when the carbon part of the molecule is used to supply the energy required and the nitrogen part is not consumed in the process of metabolism, it is left as a waste product in the form of ammonia. When there is in the soil, in addition to the proteins and protein decomposition products, a sufficient amount of available carbohydrates, the microörganisms will use the latter as a source of energy and will attack the proteins only in so far as they need nitrogen for their metabolism. In that case no ammonia will accumulate in the soil; such as is produced will probably be assimilated by the microbes. But, when there is an insufficient amount of available carbohydrates, the microörganisms are compelled to use the proteins not only as sources of nitrogen, but also as sources of energy. More carbon will then be oxidized to supply the necessary energy than there will be nitrogen consumed; the excess of nitrogen will be left in the medium as a waste product in the form of ammonia. The presence of only small amounts of available carbohydrates will check for a short period the accumulation of ammonia, but will also result in more active microbial flora The latter, after all the carbohydrate is used up, will attack the proteins present and may produce larger quantities of ammonia than if no carbohydrate had been added.

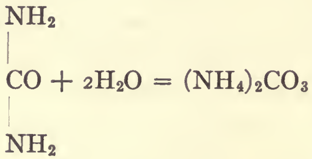
Rate of Ammonia Production.—Miyake, using the results obtained by Lipman, and Waksman, in his work on the ammonia production by *Aspergillus niger*, have shown that the rate of ammonia accumulation, whether by a pure culture or by a mixed culture, is an autocatalytic reaction. The rate of ammonia accumulation is at first slow, then it begins to fall off and finally comes to a standstill.

Relative Efficiency of Different Species.—In Marchal's experiments already referred to, the species employed showed marked differences in their ability to produce ammonia out of egg albumin. The following proportions of the protein nitrogen were converted into ammonia in twenty days:

B. mycoides	46 per cent	*B. subtilis*	23 per cent
B. (*Proteus*) *vulgaris*	36 per cent	*B. janthinus*	23 per cent
B. mesentericus vulgatus	29 per cent	*B. fluorescens putidus*	22 per cent
Sarcina lutea	27 per cent	*B. fluorescens liquefaciens*	16 per cent

Furthermore, apart from the variations from species to species, differences have been observed by Marchal and many other investigators between one strain and another of any single species isolated from the same or different soils. It must be remembered, therefore, that in the study of ammonification in soils and culture solutions, due consideration should be given to differences in *physiological efficiency* as they are manifested by strains and species of microörganisms.

Apart from the ammonifying bacteria already mentioned there is a group of organisms studied by Müller, Pasteur, van Tieghem, Leube, Miquel, Beyerinck and others. These are the so-called urea bacteria, capable of intensive transformation of urea and allied compounds into ammonium carbonate, by means of the enzyme urease.



$$\begin{matrix} NH_2 \\ | \\ CO \\ | \\ NH_2 \end{matrix} + 2H_2O = (NH_4)_2CO_3$$

Morphologicall ythese organisms include spherical and rod forms, spore-bearing and non-spore bearing species. Most of the urea bacteria are particularly prominent in the transformation of animal manures.

Ammonifying Efficiency.—Lipman and Burgess have found marked differences in the ammonifying efficiency of fifteen organisms in pure cultures using peptone, bat guano, sheep and goat manure, dried blood, tankage, cottonseed meal and fish guano. The nature of the soil as well as the nature of the nitrogenous material markedly modify an organism's ammonifying power. *B. tumescens* on the whole appears to have been the most efficient organism tested. Comparing these findings with those of Marchal the former have obtained results in soils, while the latter's work was with solution cultures, the application of which to soil conditions is not always permissible. In point of fact the ammonifying efficiency of organisms is greater in sandy soil and possibly in others than in solutions, as Lipman and Burgess have obtained a transformation of 41.98 per cent of peptone in nitrogen and 36.06 per cent of bat guano nitrogen into ammonia by *Sarcina lutea* and *B. mycoides*, respectively, in twelve days at temperatures between 27° and 30°, while Marchal obtained similar transformations in thirty days at 30° in albumen solutions.

It is also of interest to note that investigations with soil fungi have revealed the fact that certain species are even more efficient ammonifiers than *B. mycoides.* McLean and Wilson, Waksman, Coleman and Kopeloff have worked with organisms like *Trichoderma koeningi* which is capable of transforming more than 50 per cent of the nitrogenous material added in such experimentation.

NITRIFICATION. *Experimental Study.*—The term nitrification refers to the oxidation either of ammonia or of nitrites to nitrates. In a broader sense nitrification may be defined as the production of nitrates from decomposing organic matter. Saltpeter or niter, the terms formerly applied to potassium nitrate, possessed, for a long time, a peculiar interest because of its relation to gunpowder. Whether it be true or not that gunpowder was known to the Chinese before the beginning of the present era, there is no doubt that for several centuries it played an important part in the political and economic history of Europe. The large quantities of gunpowder consumed in the almost incessant wars created a steady demand for saltpeter that was not readily met by the saltpeter refiners of India, Hungary and Poland. European nations, particularly France, were therefore thrown on their own resources and were forced to develop the domestic production of saltpeter. The industry came under government control and experts were appointed to study the so-called saltpeter plantations and the conditions affecting the appearance and increase of nitrates in compost heaps and in the soil. Much knowledge was thus gained about nitrification even though it was not suspected that living organisms were concerned in the process.

With the rapid development of chemistry in the latter half of the eighteenth century a nearer approach was made to the understanding of the true character of nitrification. The observations of Cavendish in 1784 that potassium nitrate is formed when electric sparks are passed through air confined over a solution of potassium hydrate formed the starting point for various theories that attempted to account for nitrate formation on the basis of purely chemical reactions. The formation of nitric acid and of its salts was thus assumed to be due to electric discharges in the atmosphere, to combustion processes in nature, or to the oxidation of organic matter and of calcium, magnesium, iron and manganese compounds in the soil. Much credence was given to the latter explanation because of the almost universal occurrence of nitrates in arable soils.

The first indication that nitrate production in the soil and in decaying organic matter is due to biological activities was given by Pasteur in 1862. A few years later Müller expressed his belief in the biological origin of nitrates and nitrites in sewage and drinking water. It was not, however, until 1877 that the true character of nitrification was made clear. In that year Schloesing and Müntz demonstrated that dilute solutions of ammonia could be changed into nitrate by being passed slowly through long tubes filled with soil. The amounts of nitrate nitrogen found in the leachings corresponded almost exactly to the amount of ammonia nitrogen used up. When the soil in the tubes was first sterilized by heating or by means of chloroform and other germicides, the ammonia passed through unchanged. But when soils sterilized by heat or chloroform were reinfected with small quantities of fresh soils nitrification again proceeded in a normal manner.

The biological nature of nitrification having been thus established numerous investigators tried to isolate the specific organisms in pure culture. A large amount of work in this direction was done by Schloesing and Müntz, Celli and Marino-Zuco, Munro, Warington, the Franklands and many others. A large number of bacteria, yeasts and molds were tested with negative results. Warington, who gathered a great mass of valuable information about nitrification, almost succeeded in securing pure cultures of nitrifying bacteria. Finally, Winogradski showed in 1890 not only that nitrification is caused by specific bacteria, but explained also why the others failed in securing pure cultures. He proved that these organisms do not develop colonies on the ordinary gelatin and other organic media, a fact whose recognition was largely responsible for his successful solution of the problem. The medium subsequently employed by him consisted of silica jelly properly supplied with inorganic nutrient salts. After him other investigators proved that agar, deprived of its soluble organic matter, gypsum and sandstone disks, filter-paper pads, etc., could be used effectively as solid media.

Nitrous and Nitric Bacteria.—Winogradski's investigations led to the conclusion, foreshadowed by the earlier work of the Franklands and Warington, that the oxidation of ammonia proceeds in two stages, viz.,

$$(1)\ 2NH_3 + 3O_2 = 2HNO_2 + 2H_2O$$

$$(2)\ 2HNO_2 + O_2 = 2HNO_3$$

The organisms oxidizing ammonia to nitrites, and designated as nitrous or nitrite bacteria, were called by Winogradski *Nitrosomonas* and *Nitrosococcus*. The former include species or varieties isolated from soils in Europe, Asia and Africa, and the latter those isolated from soils in America and Australia. The organisms oxidizing nitrites to nitrates and known as nitric or nitrate bacteria, were included by Winogradski in the genus *Nitrobacter*.

Apart from these bacteria there is an organism, according to Kaserer, that can oxidize ammonia directly to nitrate. He named it *B. nitrator*. The reaction is illustrated by the following equation:

$$NH_3 + H_2CO_3 + O_2 = HNO_3 + H_2O + CH_2O - 41 \text{ Cal.}$$
$$CH_2O + O_2 = H_2CO_3 + 132 \text{ Cal.}$$

Enough energy for the completion of the reaction is obtained by the oxidation of the formaldehyde (CH_2O). Beyond the preliminary announcement of Kaserer's there are no experimental data to prove the existence of this organism, even though other evidence of an indirect nature may be construed to lend support to his theory. But whether it be proved or not that ammonia may be oxidized to nitrate by a single species, it is evident that the number of species concerned in nitrate production is relatively small.

Relation to Environment.—The conditions that affect nitrate formation in soils may be classified under the following heads: (*a*) supply of oxygen; (*b*) range of prevailing temperatures; (*c*) amount and distribution of moisture; (*d*) quantity of lime and of other basic materials; (*e*) quantity of soluble mineral salts; (*f*) character and amount of organic matter; (*g*) presence of toxic substances; (*h*) association with other organisms; (*i*) physiological efficiency of the nitrifying bacteria.

The rapid disappearance of organic matter from sandy soils is due in large measure to their better aeration. On the other hand, the decomposition of vegetable and animal substances in heavy, ill-ventilated soils is materially retarded by the limited supply and very gradual renewal of oxygen. An intimate relation exists here between air and water in that the latter replaces the former to a more marked extent in heavy than in light soils. The influence of both aeration and the range of moisture is illustrated by an experiment of Lipman's in which equal quantities of soil were kept in large boxes under different moisture conditions. At the end of a year the following quantities of nitrate nitrogen were found:

Moisture content	6.52 per cent	14.75 per cent	18.62 per cent	22.05 per cent	22.12 per cent
Nitrate nitrogen found	697 mg.	823 mg.	720 mg.	Trace	Trace

In examining the figures recorded above, we find that moisture was the controlling factor in the development of the nitrifying bacteria, when the proportion of water in the soil was 6.52 per cent. As the amount of water increased to 14.75 per cent there was a marked increase in the amount of nitrate produced. Beyond that, however, the further increase in the amount of water began to limit the supply of oxygen, and the production of nitrate nitrogen with 18.62 per cent of water in the soil was somewhat decreased. A still further addition of water up to 22.05 per cent led, practically, to saturation, and the encouragement of reduction rather than oxidation processes. Hence, no nitrate was allowed to accumulate in the soil. The data in question thus help to explain why care was taken, on saltpeter plantations, to keep the compost heaps moist, yet not too wet.

The influence of temperature on nitrate formation has been observed by many investigators. Schloesing and Müntz recorded that at 5° nitrification is quite feeble, at 12° marked and at 37° at its best. Other investigators have obtained substantially the same results, except that the optimum has been found to be considerably lower, often between 25° and 30°. Under field conditions nitrification seems to take place at relatively low temperatures, as is indicated by the rapid oxidation of ammonium salts in the Rothamsted experiments in England; and the rapid decay and nitrification of clover and of other legume residues in the experiments at the New Jersey Experiment Station. These facts have, therefore, an important bearing on the nitrogen feeding of crops in tropical, subtropical and temperate zones.

The influence of lime and of other basic substances including the carbonates of magnesium, potassium and sodium, and of the oxides of iron is of far-reaching importance in all nitrification processes. It is well known that applications of magnesian and non-magnesian lime, marl or wood ashes promote nitrification in the soil and in compost heaps, a fact that was well recognized by the ancient niter refiners. The favorable action of lime is readily explained by its ability to neutralize organic and mineral acids and to render, thereby, the soil reaction favorable for the rapid growth of ammonifying, as well as of nitrifying bacteria. Furthermore, the reserve of basic material serves to neutralize the acid formed by the bacteria and prevents thus the accumulation of an undue amount of acidity.

The rôle of certain mineral salts in promoting nitrification is quite significant. Small amounts of sodium chloride have been found to favor nitrification in the experiments of Pichard and also those of Lipman. The former showed also that sulphates not only promote nitrification, but that different sulphates display marked variations in this respect. In the same manner nitrate formation was shown to be favorably affected by phosphates in bone meal, Thomas slag, and acid phosphates. Generally speaking, therefore, nitrifying bacteria are stimulated in their development by a proper supply of available mineral nutrients.

The exact relation of organic matter in the soil to the activities of nitrifying bacteria is but beginning to be properly understood. Earlier observations made it manifest that heavy applications of animal manures, or of green manure may not only retard nitrification, but may actually cause the disappearance of a part or of all of the nitrate in the soil. Subsequent experiments by Winogradski and by Winogradski and Omelianski showed that in pure cultures the presence of even slight amounts of soluble organic matter may depress or even suppress the development of the nitrifying bacteria. It was, therefore, concluded by these authors that relatively small amounts of soluble organic matter may inhibit nitrification. These conclusions, based on the study of liquid cultures only, were given a very broad application by many writers on agricultural topics. More recent experiments make it certain, however, that in the soil itself small amounts of soluble organic matter, *e.g.*, dextrose, are not only harmless, but may really stimulate nitrification. It was shown, likewise, that humus and extracts of humus may, under suitable conditions, stimulate nitrification to a very striking extent.

Certain substances in the soil may exert a toxic effect on nitrifying bacteria. Ferrous sulphate, sulphites and sulphides may thus act injuriously, as may also calcium chloride and excessive concentrations of sodium carbonate, sodium bicarbonate, sodium chloride, magnesium sulphate, etc. Injury by ferrous compounds, as well as by organic acids, is not uncommon in low-lying fields and bogs; while injury from excessive concentration of soluble salts may occur in the so-called alkali lands.

Finally nitrification in the soil should be considered from the standpoint of the organisms themselves. There is no doubt that continued growth under extremely favorable conditions leads to the development in the soil of nitrifying bacteria, possessing a very marked physiological efficiency. On the other hand, in ill-aerated, sour soils the environment would depress the physiological efficiency of the nitrifying bacteria. Differences are thus undoubtedly established under actual field conditions, as is made probable by the variable behavior of soils from different sources when used as inoculating material in recently reclaimed or peat swamp lands.

Accumulation and Disappearance of Nitrates.—As shown above, the rate of formation of nitrates in the soil is dependent upon moisture, temperature and aeration, as well as on the presence of organic matter and basic substances. On the other hand, the accumulation of nitrates depends, under any given conditions, largely on the character of the growing crop. Observations on the rain gauges at Rothamsted showed an average annual loss of 14 kg. (31.4 pounds) of nitric nitrogen per acre in the drainage water from uncropped soil. In one of King's experiments, land that had been fallowed contained 137 kg. (303.24 pounds) of nitric nitrogen per acre, to a depth of 4 feet. Adjoining cropped land contained only 26 kg. (57.56 pounds) of nitric nitrogen per acre to the same depth. Stewart and Greaves found in limestone soil in Utah 64 kg. (142 pounds) of nitric nitrogen per acre, under corn; 98 pounds under potatoes, and only 12 kg. (27 pounds) under alfalfa. Under the same conditions fallow land contained 74 kg. (165 pounds) of nitric nitrogen per acre. The smaller amount of nitric nitrogen found under alfalfa bears out the observations already made by a number of other investigators that the accumulation of nitrates under legumes is smaller than it is under non-legumes. While several explanations have been offered to account for this fact, it is generally agreed that legumes assimilate nitrate nitrogen more rapidly than non-legumes. Unusual circumstances may favor, at times, the accumulation of quantities of nitrate large enough to destroy all vegetation. It is reported, for instance, by Headden that he has found in limited areas in Colorado as much as 90,718.5 kg. (100 tons) of nitrate per acre foot of soil.

The amount of nitrate nitrogen in the soil is influenced by the growing crop not alone because of the nitrogen absorbed by the latter, but because of the moisture relations as affected by growing plants. It is quite apparent that a large crop dries out the soil more rapidly than a small crop. When the soil moisture is sufficiently depleted, nitrification stops and the further accumulation of nitrates becomes impossible, while their disappearance is hastened by the constant demands of the crop. The disappearance of soil nitrates is, likewise, hastened by the leaching action of rain and by certain species of bacteria that transform them into other nitrogen compounds.

DENITRIFICATION. *Experimental Study.*—Denitrification may be defined as the reduction of nitrates by bacteria, involving the evolution of nitrogen gas or of nitrogen oxides. In a more general way, denitrification has been defined as the partial or complete reduction of nitrates by bacteria. The term direct denitrification has been suggested for complete reduction, and indirect for the partial reduction to nitrites or ammonia. The term denitrification should not be employed to designate losses of nitrogen gas due to the oxidation of ammonia, or to the disappearance of nitrates following their conversion into proteins by microörganisms.

The reduction of nitrates in the presence of fermenting organic matter was noted by Kuhlmann as early as 1846. The same fact was recorded many years later by Froehde and by Angus Smith. In 1868 Schoenbein expressed the belief that nitrates may be reduced to nitrites by fungi. For more than a decade after that, data were rapidly accumulating in support of Schoenbein's contention, until in 1882 Gayon and Dupetit made it certain that nitrate reduction with the evolution of nitrogen gas may be caused by a "ferment." Finally, in 1886, the same investigators described two organisms, *B. denitrificans α*, and *B. denitrificans β*, capable of completely reducing nitrates. Subsequently the studies of Giltay and Aberson, Burri and Stutzer, Severin, van Iterson, Jensen, Beyerinck and of many others not only greatly increased the number of known denitrifying bacteria, but added much to our knowledge concerning the development and activities of these organisms. It has been shown that a very large number of species can reduce nitrates to nitrites and ammonia: moreover, a considerable number of organisms are already known that can cause the complete destruction of nitrates with the evolution of nitrogen gas or nitrogen oxides. The following reactions illustrate diagrammatically the complete or partial reduction of nitrates.

$$2HNO_3 = 2HNO_2 + O_2$$
$$HNO_3 + H_2O = NH_3 + 2O_2$$
$$4HNO_2 = 2H_2O + 2N_2 + 3O_2$$

In the soil, manure or other culture media the denitrifying bacteria which are, for the most part, aerobic develop also under anaerobic conditions and transfer the oxygen of nitrates and nitrites to carbon compounds. This is illustrated by the equations suggested by van Iterson:

$$5C + 4KNO_3 + 2H_2O = 4KH\ CO_3 + 2N_2 + CO_2$$
$$3C + 4KNO_2 + H_2O = 2KH\ CO_3 + K_2CO_3 + 2N_2$$

When nitrates are reduced to nitrites in the presence of amino-compounds, or even of ammonium compounds, elementary nitrogen may escape as shown by the following reactions:

$$C_2H_5NH_2 + HNO_2 = C_2H_5OH + N_2 + H_2O$$
$$NH_4Cl + KNO_2 = KCl + 2H_2O + N_2$$

An organism has been described by van Iterson that can decompose nitrates in the presence of cellulose:

$$5C_6H_{10}O_5 + 24KNO_3 = 24KHCO_3 + 6CO_2 + 12N_2 + 13H_2O$$

Still more interesting is *Thiobacillus denitrificans* described by Beyerinck as capable of reducing nitrates in inorganic media. The nitrate oxygen is used to oxidize elementary sulphur:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

The *Actinomyces* reduce nitrates to nitrites, but they do not cause any loss of free nitrogen, for the nitrites are utilized by the organisms. and complete denitrification does not take place. Thus these organisms may prevent the leaching out of nitrates and nitrites in the soil, or the active denitrification by other organisms.

Relation to Environment.—Nitrate reduction is favored by insufficient aeration, as well as by an abundance of readily decomposable organic matter. In fine-grained, compact soils nitrate formation and nitrate reduction may alternate, depending upon the more or less complete replacement of soil air by water. Similarly, in soils receiving excessive amounts of animal manure denitrifying bacteria may cause the reduction of nitrates. In greenhouse soils excessive moisture, as well as excessive amounts of organic matter, may be present and may prevent the accumulation of nitrates. It has also been shown by Niklevski that, contrary to opinions previously held, denitrification may occur in manure heaps. In the better aerated surface portion of manure heaps conditions are favorable for the oxidation of ammonia to nitrites and nitrates. The nitrous acid may combine with ammonia to form ammonium nitrite, the latter decomposing, spontaneously, into water and nitrogen gas. It is very likely, also, that the nitrites and nitrates are reduced by the denitrifying bacteria in manure. On the other hand, in manure kept moist under the feet of cattle nitrite and nitrate formation is prevented and losses by denitrification are not likely to occur.

The economic significance of denitrification was overestimated at one time, on account, largely, of the assertion of Wagner in 1895 that in all soils receiving applications of horse manure, the nitrates in the soil itself as well as those added in commerical fertilizers are almost certain to be destroyed by denitrification. Subsequent experiments by many investigators demonstrated that under field conditions, denitrification is a factor of slight moment; however, in the greenhouse, in the manure heap (under certain conditions) and in market gardening where manure is used at the rate of 45,359 kg. to 54,431 kg. (50 to 60 tons) per acre, the danger of denitrification is real.

ANALYTICAL AND SYNTHETICAL REACTIONS

AMOUNT OF BACTERIAL SUBSTANCE IN THE SOIL.—Various decomposition processes in the organic matter of the soil may be designated as analytical in that protein, carbohydrates and fats are split into more simple compounds. At the same time, the microörganisms concerned in the decomposition processes multiply very rapidly and fashion the complex compounds of their cell-substance out of the simple cleavage products in their medium. In other words, analytical and synthetical reactions proceed hand in hand in the soil.

While it is not definitely known how large a proportion of the soil humus consists of the dead and living cells of microörganisms there is a mass of indirect evidence to show that these cells form a very considerable proportion of the total quantity of organic substances in the soil. For instance, it has been demonstrated that a large proportion of the dry matter of solid animal fæces may consist of bacterial cells. At various times and by different investigators the proportion of bacterial substance has been estimated at from 5 to 20 per cent or more of the total dry weight of fæces. A heavy application of barnyard manure may introduce, therefore, several hundred pounds of bacterial cells per acre of soil. Moreover, because of the extensive changes in the soil humus itself, as is evidenced by the rapid formation of nitrates, large masses of bacterial substances are constantly being formed and disintegrated.

AVAILABILITY OF BACTERIAL MATTER.—Substances of microörganic origin are decomposed more or less rapidly, according to their composition. The extent of transformation under favorable conditions is indicated by an experiment performed by Beyerinck and van Delden, in which 50 per cent of the nitrogen in *Azobacter* cells was transformed into nitrate in seven weeks. On the other hand, the humus of peat and muck soils, or that of worn-out soils, may contain microörganic residues of so inert a character as to yield but little available nitrogen to crops.

TRANSFORMATION OF PEPTONE, AMMONIA AND NITRATE NITROGEN. The cleavage of protein compounds into peptones, amino-acids and ammonia, and the oxidation of the latter into nitrites and nitrates, may be properly included among analytical reactions. It should not be forgotten, however, that in the accompanying synthetical reactions the compounds just mentioned may be transformed back into complex proteins. It happens, thus, that large quantities of the available nitrogen compounds may be withdrawn from circulation by microörganisms that use these as building material. Under extreme conditions microörganisms may become serious competitors of higher plants for available nitrogen food.

Manure stored in heaps not infrequently deteriorates in quality, even when losses by leaching are excluded. This deterioration is largely due to the change of the water-soluble ammonia and amino-compounds into insoluble protein substances. While the extent of the change into protein compounds is variable it may range from less than a tenth of the water soluble material to more than three-quarters or four-fifths of it. Also in the soil the same processes take place, but not so intensively. A large number of species of molds and bacteria have been isolated and tested as to their ability to transform ammonia, amino- and nitrate nitrogen into protein compounds. Among the more recent investigations in this field those of Lemmermann and his associates testify that in three weeks 5 to 6 per cent of the nitrate added to the soil was changed into protein. In the presence of barnyard manure the proportion transformed was increased to 15 per cent. In the case of ammonium compounds the transformation may be even more far-reaching, amounting, at times, to more than 25 to 30 per cent of the material originally present. Generally speaking, molds will assimilate ammonia nitrogen more readily while bacteria and algæ will assimilate nitrate nitrogen by preference. However, the preference of molds for ammonia nitrogen is often more apparent than real, because of the rapid formation of acid residues in culture media rich in certain ammonium compounds. Similarly, some species of bacteria will assimilate ammonia nitrogen in preference to nitrate nitrogen.

CHAPTER III

FIXATION OF ATMOSPHERIC NITROGEN

The Source of Nitrogen in Soils

Early Theories.—When chemistry had made sufficient progress to allow the analysis of soils and plants it was recognized that nitrogen is always present in both. It was also recognized that the soil nitrogen is almost wholly confined to the surface portion and is evidently of atmospheric origin, since the unweathered, underlying rock is devoid of this constituent. The vast accumulations of nitrogen, known to exist in all arable soils, were ascribed, therefore, to the residues of many generations of plants; and the assumption seemed to be justified that the atmosphere, 79 per cent of whose bulk consists of nitrogen gas, is the direct source of this element to plants. It was not long, however, before plant physiologists demonstrated experimentally that nitrogen gas as such could not directly serve as food for plants. There thus arose one of the most interesting and, for a long time, one of the most puzzling problems in agricultural research. Among the earlier investigators de Saussure believed, at the beginning of the nineteenth century, that nitrogen is taken up from the soil in combined form. Liebig in 1840 advanced his well-known "mineral theory" according to which plants secured their nitrogen from the air, in the form of ammonia. He assumed, thus, that plants cannot use elementary nitrogen, and that the supply of atmospheric nitrogen in the form of ammonia was great enough to meet the needs of growing vegetation. The latter view was not accepted by Lawes and Gilbert of the Rothamsted Station in England. By a series of elaborate and carefully controlled experiments they demonstrated in 1858 that nitrogen in the elementary form cannot be used by plants. They further demonstrated that the amount of combined nitrogen brought down in the form of ammonia, nitrites and nitrates, by atmospheric precipitation was but slight when compared with the quantities annually removed by crops. Hence the problem as to the source and maintenance of combined nitrogen in the soil seemed to be more puzzling than ever.

CHEMICAL AND BIOLOGICAL RELATIONS.—The second and third quarters of the nineteenth century saw the birth of a number of theories dealing with this problem. It was suggested that nitrogen compounds may be formed in the soil by the oxidation of nitrogen to nitric acid. Compounds of iron, manganese and lime were supposed in some way to make such oxidation changes possible. It was likewise suggested that nascent hydrogen may be generated in the decomposition of organic matter in the soil, and reacting with elementary nitrogen, may give rise to ammonia. The various hypotheses were not supported by experimental proof; moreover, the situation was complicated by the knowledge, based on empirical observations, that crops of the legume family seemed to be more or less independent of the supply of combined nitrogen in the soil. Indeed, clovers and other legumes had, apparently, the ability to increase the content of combined nitrogen in the soil as was indicated by the experiments of Boussingault and of Lawes and Gilbert. Finally, the mystery was solved by the investigations of Berthelot and Hellriegel and Wilfarth who furnished the proof that elementary nitrogen may be utilized by plants when certain biological relations are met. These relations involve the presence and activities of microörganisms that by themselves, or in conjunction with higher plants, make available to growing vegetation the great store of atmospheric nitrogen.

NON-SYMBIOTIC FIXATION OF NITROGEN

HISTORICAL.—Non-symbiotic nitrogen fixation, or *Azofication*, has already been defined as the production of nitrogen compounds out of atmospheric nitrogen by bacteria independently of higher plants. The part played by bacteria in this process was not recognized until 1885, when Berthelot published some of his data on the accumulation of combined nitrogen in uncropped soils. His results seemed to explain a number of scattered observations, made since the middle of the century, on the apparent increase of the nitrogen content of cultivated soils.

While Berthelot's experiments proved that the nitrogen gains occurred only in unsterilized soils and were, therefore, due to microorganisms, it remained for Winogradski to demonstrate, in 1893, that the formation of nitrogen compounds by certain types of bacteria may be accomplished in culture media nearly or quite devoid of combined nitrogen. Soon after that he succeeded in isolating his organisms in pure culture, and described them as anaerobic bacilli allied to those of the butyric group. In 1901 our knowledge of *Azobacteria* was enriched by Beyerinck's discovery of a group of large, obligate aerobic bacteria that he designated as *Azotobacter.* Since that date it has been found that the ability to fix atmospheric nitrogen is possessed also by certain molds and by various species of bacteria. However, this ability is not only extremely variable, but is also very feeble as compared with that of the members of the two groups described by Winogradski and Beyerinck. These two groups may, therefore, be designated as including the nitrogen-fixing bacteria par excellence.

ANAEROBIC SPECIES.—The species isolated by Winogradski was named by him *B.* (*Clostridium*) *pasteurianus* (Fig. 131). It was found to grow readily under anaerobic conditions in culture solutions containing dextrose and the necessary mineral salts, but no combined nitrogen. The products of growth included protein, butyric and acetic acids, carbon dioxide and hydrogen. In the presence of other bacteria *B.* (*Clostridium*) *pasteurianus* was found to develop also under aerobic conditions. Subsequently studies by Winogradski and other investigators showed that *B.* (*Clostridium*) *pasteurianus*, and varieties of this species are very widely distributed in cultivated soils. More recently Bredeman made a thorough and extended study of anaerobic *Azobacteria* and demonstrated their almost invariable presence in a large number of soil samples from Europe, Asia and America. In his opinion they correspond more or less closely to *B. amylobacter* described many years before by van Tieghem.

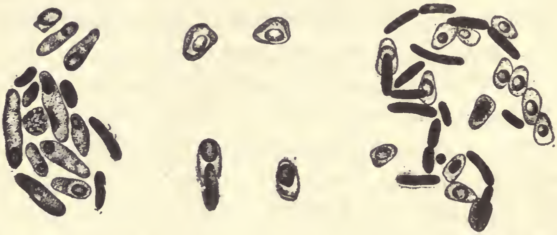


FIG 131.—*B.* (*Clostridium*) *pasteurianus*, a non-symbiotic nitrogen-fixing organism. (*After Winogradski from Lipman.*)

AEROBIC SPECIES.—A more or less pronounced power to fix atmospheric nitrogen is apparently possessed by a considerable number of aerobic species. Lipman has demonstrated the fixation of small amounts of nitrogen by *Ps. pyocyanea* and Löhnis secured similar results with *Bact. pneumoniæ, B. lactis viscosus, B. radiobacter* and *B. prodigiosus.* Gottheil has detected fixation by *B. ruminatus* and *B. simplex;* Pillai has described a nitrogen-fixing aerobic bacillus, *B. malabarensis;* Westermann studied a similar organism that he named *B. danicus;* while Beyerinck and van Delden observed, some years earlier, that certain strains of *B. mesentericus* could fix relatively large amounts of nitrogen. Similarly *Ps. radicicola* has been found to possess a slight, but nevertheless an appreciable power to fix elementary nitrogen in culture solutions or in the soil.

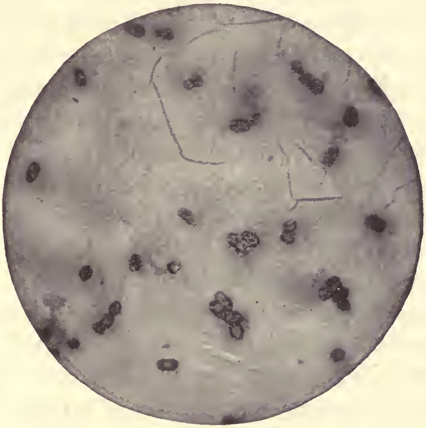


FIG. 132.—*Azotobacter vinelandi,* a non-symbiotic nitrogen-fixing organism. (*After Lipman.*)

But while nitrogen fixation among aerobic soil bacteria is not as uncommon as was at one time supposed, this function is so feeble and so variable in most instances, as to be of negative, or, at best, of doubtful economic significance. On the other hand, the aerobic, *Azotobacter,* first described by Beyerinck in 1901, may be regarded not only as possessing a very pronounced ability to fix atmospheric nitrogen, but as playing a rôle of some moment in maintaining the supply of combined nitrogen in the soil.

To the two species of *Azotobacter, A. chroococcum* and *A. agilii* described by Beyerinck and van Delden, Lipman added *A. vinelandis*

(Fig. 132), *A. beyerincki* and *A. woodstownii*, and Löhnis and Westermann, *A. vitreum*. Of these species *A. chroococcum* and *A. beyerincki* are most common and are widely distributed in cultivated soils of Europe and America, and probably also of the other continents. They are absent in acid soils deficient in humus, and most common in limestone regions and in irrigated soils rich in mineral salts. Their food requirements are covered by solutions containing potassium phosphate, magnesium sulphate, calcium chloride and ferric sulphate, and some organic nutrient, such as dextrose, saccharose, xylose, mannit, acetate, propionate, butyrate, malate, ethyl alcohol, etc. An alkaline or neutral reaction and the presence of salts of iron are essential for the vigorous development of *Azotobacter*, while humates have been shown by Krzemieniewski to exert a stimulating influence on the growth of these organisms, even though not acting directly as a source of food and energy. As shown by Lipman and others, *Azotobacter* may gain an increased power of fixing atmospheric nitrogen in the presence of other organisms. It is resistant to drying, notwithstanding the fact that it produces no spores, and has been successfully isolated from soil samples that had been kept in a dry state for several years. For some reason it may be detected in the soil most readily in the fall and winter months.

As to the nitrogen-fixation by fungi, it has been shown elsewhere that the evidence is, if anything, of a negative character. Some algæ are able to fix atmospheric nitrogen, especially those that live symbiotically with azotobacter.

Energy Relations.—In the fixation of nitrogen by bacteria the necessary energy for the process is furnished by the carbohydrates, organic acids, alcohols or other organic nutrients employed in the culture media. Since any given quantity of organic nutrient possesses a definite amount of potential energy the fixation of nitrogen is necessarily limited by the supply of such potential energy. This limitation was already recognized by Winogradski in his experiments with *B. (Clostridium) pasteurianus*. For every gram of dextrose used up there was produced, on the average, 2 to 3 mg. of combined nitrogen. In the experiments of Bredeman with *B. amylobacter*, and of Pringsheim with "*Clostridium americanum*" the amounts fixed were, at times, considerably larger. On the whole, however, it has been proved by a number of investigators that *Azotobacter* can fix much larger quantities of nitrogen than the anaerobic bacilli. The extended investigations of Lipman showed that *A. vinelandii* has the ability to fix more nitrogen per unit of organic nutrient consumed than either *A. chroococcum* or *A. beyerincki.* Under favorable conditions *A. vinelandii* may at times fix 15 or even 20 mg. of nitrogen per g. of mannit used up. Krzemieniewski found in experiments with *A. chroococcum* that additions of humates to the culture solutions increased the nitrogen fixed from a maximum of 2.4 mg. to a maximum of 14.9 mg.

The practical bearing of the foregoing data lies in the fact that the fixation of nitrogen in cultivated soils is limited, among other things, by the energy available, that is, by the quantity of readily decomposable organic residues. An indication as to the extent of these is given by the amount of humus present; nevertheless, this must remain an indication merely, for most of the humus is too inert to serve as a source of energy to *Azotobacter.* From the data at present available different investigators have estimated the quantity of nitrogen fixed by *Azotobacter* at 6.8 kg. to 18 kg. (15 to 40 pounds) per acre, per annum. Assuming favorable conditions for fixation, so that 500 g. (1 pound) of nitrogen could be fixed for every 50 kg. (100 pounds) of carbohydrate consumed, it would still take an equivalent of 680 kg. to 1,814 kg. (1,500 to 4,000 pounds) of sugar to produce this quantity of combined nitrogen. It may be noted in this connection that *Azotobacter* have been demonstrated to live in symbiosis with algæ, obtaining thereby the necessary energy for their activities. This may explain, perhaps, the remarkable facts observed by Headden in Colorado, relating to the accumulation of such enormous quantities of nitrate in the soil as to destroy all vegetation. In some instances the nitrates were found to be present to the extent of 90,718 kg. (100 tons), or more (per acre), to a depth of a few inches. If the accumulation of combined nitrogen was due to *Azotobacter*, as is claimed by Headden, and the bacterial residues oxidized by nitrifying bacteria to nitrates, it is difficult to account for the source of the 1,000 or 2,000 tons of carbohydrates necessarily used up in the process of fixation, unless it could be proved that the energy was furnished by algæ.

SYMBIOTIC FIXATION

HISTORICAL.—Empirical observations extending well back into ancient agriculture have led to the recognition of the soil-enriching qualities of certain crops of the legume family. Columella mentions the fact that many Roman farmers regarded beans as possessing these qualities, but does not accept this belief for himself. On the other hand, he points out that luzerne (alfalfa), lupins and vetches improve the land and act as manure. He points out, also, that it was the practice of Roman farmers to plow under lupines in order to enrich the soil. In the centuries following the fall of Rome the use of legumes for soil improvement persisted to some extent in Italy, France and other countries; yet the practice was not followed consistently and the fertility of European soils was declining for lack of available nitrogen, and, to a large extent, also of phosphoric acid. The more general introduction of clover into Germany and England in the eighteenth century helped to restore the fertility of many farms, and led, ultimately, to the recognition of the peculiar place held by legumes in the maintenance of soil fertility. But while practical farmers knew of the soil-enriching power of legumes, and while they retained their belief in it even when it seemed contrary to scientific authority, they did not know the secret of this power. It remained for Hellriegel and Wilfarth to demonstrate in 1886, and more fully in 1888, that this power, already hinted at by the investigations of others, is the resultant of the combined activities of the plants and of bacteria that enter their roots, and produce there the well-known nodules or tubercles. They showed in no uncertain manner that legumes can improve the soil only in so far as they add nitrogen to it with the aid of the bacteria in the tubercles; in other words, legumes were shown to enter into a symbiotic relationship with certain bacteria and to acquire, thereby, the ability to fix atmospheric nitrogen.

The presence of tubercles on the roots of leguminous plants was first recorded by Malpighi in 1687. He regarded them as root galls. The botanists who studied them in the first half of the nineteenth century classified them as modifications of normal roots or as pathological processes. In 1866 the Russian botanist Woronin found that the tubercles were filled with minute bodies resembling bacteria and concluded that they were pathological outgrowths. Some years later Frank, in 1879, not only showed that tubercles are almost invariably present on the roots of legumes, but that their formation may be prevented by sterilizing the soil. Frank was thus in possession of facts that might have revealed to him the true nature of the root-tubercles.

However, he later modified his belief in the origin of tubercles as due to outside infection, and accepted the interpretation of his pupil Brunchhorst who claimed that the bacteria-like bodies in the tubercles were merely reserve food materials. Because of their resemblance to bacteria Brunchhorst named them bacteroids.

The studies of Marshall Ward, published in 1887, proved not merely that tubercle formation is due to outside infection, but that such infection may be brought about at will by placing the roots of young plants in contact with pieces of old tubercles. Hellriegel in his preliminary communication of 1886 also showed that outside infection is necessary for the production of tubercles, and called attention to the true function of the latter as laboratories wherein nitrogen compounds are manufactured out of elementary nitrogen. The true worth of Hellriegel's investigations was brought out more clearly in another paper that he published jointly with Wilfarth in 1888. The authors showed that in sterilized soils legumes behaved precisely like non-legumes, and died ultimately of nitrogen hunger when not provided with nitrates or other suitable nitrogen compounds. On the other hand, when the sterilized soil was later infected with a few drops of leachings from fresh soil that had supported a normal growth of legumes, the starving plants recovered and grew vigorously. Under the same conditions non-legumes did not recover. The recovery of the starving legumes was found to be coincident with the formation of tubercles.

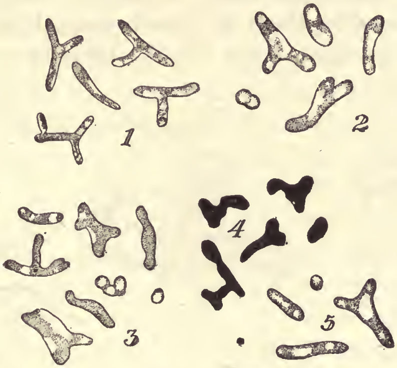


FIG. 133.—*Ps. radicicola.* 1, From *Melilotus alba;* 2 and 3, from *Medicago sativa.* 4, from *Vicia villosa.* (*After Harrison and Barlow from Lipman.*)

Hellriegel and Wilfarth's studies were soon confirmed by the investigations of others. Wigand showed in 1887 that the tubercles contained within them true bacteria. In the following year Beyerinck reported the successful isolation of these bacteria on artificial media, and named the organism *B. radicicola* (Fig. 133). Prazmowski also isolated pure cultures of *Ps. radicicola*, and followed the entrance of the organisms into the root hairs of young plants, their passage through the cell-walls, and their transformation into bacteroids. These facts were all confirmed by other investigators, and it was further shown by Schloesing and Laurent that properly inoculated legumes not only can grow in soils devoid of combined nitrogen, but that when growing in such soils in a confined atmosphere they decrease the quantity of nitrogen gas surrounding them by transforming it into nitrogen compounds. It was, therefore, made clear by these investigations, and by others not mentioned here for lack of space, that the belief of practical farmers in the soil enriching qualities of legumes was amply justified. It was shown, further, that the later experiments of Boussingault, as well as those of Lawes, Gilbert and Pugh failed to solve the problem because these investigators treated their soil so as to prevent the survival and subsequent entrance of *Ps. radicicola*, and deprived the leguminous plants of the ability to utilize atmospheric nitrogen.

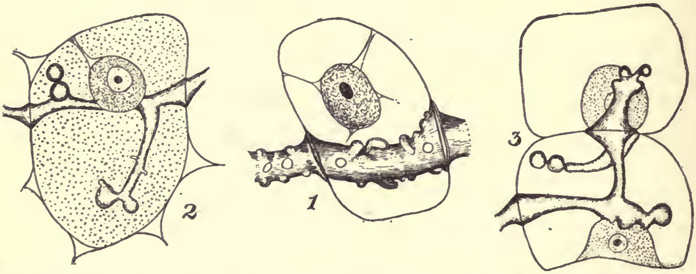


Fig. 134.—Sections through root tubercles. 1, Cell from tubercle of *Pisum sativum*, showing bacterial filament; 2 and 3, cells with bacterial filaments from tubercle of *Trifolium pannonicum*. (*After Stefan from Lipman.*)

Modes of Entrance and Development.—Tubercle bacteria consisting of small motile rods usually enter the legumes by way of the root-hairs. For this reason young tubercles, with but few exceptions, are found on young roots. The organisms multiply at the point of infection and penetrate into adjacent plant-tissue by means of a hypha-like hollow thread or tube that seems to consist of a gelatinous material (Fig. 134). The tubes branch out as they pass from cell to cell and carry the invading organisms with them. The bacteria which may be readily detected within the tubes and cells are the involution forms of *Ps. radicicola* and assume various irregular shapes. They are designated as bacteroids. Stefan has suggested that bacteroids may be produced within the tubes and, possibly, from the buds or swellings that appear on the tubes. While still young, the bacteroids are capable of dividing, but as they grow they swell up and finally degenerate.

RESISTANCE, IMMUNITY AND PHYSIOLOGICAL EFFICIENCY.—The invasion of legumes by *Ps. radicicola* and the acquisition by the plant, thanks to this invasion, of the power to fix elementary nitrogen are cited as a case of symbiosis. However, some writers would regard the presence of *Ps. radicicola* in legume roots as a case of parasitism. According to them symbiosis presupposes the living together of two organisms with resulting benefit to both. In the present instance, however, conditions may arise when the host plant is injured, rather than benefited; and similarly, conditions may arise when the invading bacteria are suppressed by the plants. Making due allowance for the objections raised we still find, nevertheless, that in the broad relation of the two groups of organisms there is an apparent benefit to both plants and bacteria. The former gain an adequate supply of nitrogen and the latter a supply of carbohydrates and of mineral salts.

A more detailed study of this relation shows that the plants resist the entrance of bacteria. When an abundance of available nitrogen compounds is supplied tubercle formation may be largely or wholly suppressed. In that case the plants secure their nitrogen from the soil and are not only independent of the bacteria, but are strong enough to resist their entrance. It is further claimed by Hiltner that tubercle bacteria differ in their virulence, and that the more virulent the organisms, the more readily will they penetrate the root tissue. Moreover, he believes that when a plant is invaded by organisms of any degree of virulence, the host plant becomes immune to a large extent and can keep out all but the most virulent bacteria. The use of the term virulence, in this connection, has been objected to, since it is borrowed from animal pathology and is likely to be misleading. It is better to employ the term *physiological efficiency* as implying not only a more pronounced ability to enter the plant roots, but also to fix atmospheric nitrogen. It is conceivable that strains of *Ps. radicicola* may be developed that would grow rapidly and yet possess but a feeble nitrogen-fixing power. In other words, they would possess a high vegetative power and a low physiological efficiency.

Mechanism of Fixation.—It is generally believed that the fixation of nitrogen is accomplished by the bacteria within the tubercles. The claim, at one time, advanced by Stoklasa, that the fixation is accomplished by the plants themselves with the aid of enzymes produced by the bacteria in their roots, has been disproved. It is known that the period of active nitrogen assimilation by the plants coincides with the appearance of the bacteroids in the tubercles, and it is supposed that the microörganisms fashion nitrogen compounds out of atmospheric nitrogen by using the carbohydrates and organic acids in the plant juices as a source of energy. The plants then seem to utilize the soluble nitrogen compounds that pass out of the bacterial cells. It is further supposed that bacteroid formation is an attempt on the part of the microörganisms to adjust themselves to the drain caused by the activities of the host plant.

Variations and Specialization.—Apparent differences in bacteria from different legumes were noted by Hellriegel. Some of his experiments indicated that bacteria from clovers could not produce tubercles on lupines and serradella. Analogous differences were found by Nobbe and his associates, nevertheless they were finally led to conclude that the root invasion of legumes is caused by a single species. However, continued association with any particular legume accomplished in the end a certain modification, or specialization, as it were, of the microörganisms, and they were then no longer able to invade the roots of other legumes. Later, Hiltner and Störmer have been led to modify this view and have arranged the tubercle bacteria in two groups, possessing, according to them, well-defined morphological and physiological differences. One of these groups is included under the species "*Rhizobium radicicola*" and the other under "*Rhizobium beyerinckii*." The former comprises the organisms from lupines, serradella and soy beans while the latter comprises all of the others.

Relation to Environment.—Nitrogen fixation by leguminous vegetation is readily influenced by soil conditions, particularly the supply of lime and of other basic substances; the supply of organic matter and the aeration of the soil. As to the first of these it is well known that all legumes, with the exception of lupines and serradella, are stimulated in their growth by generous applications of lime.

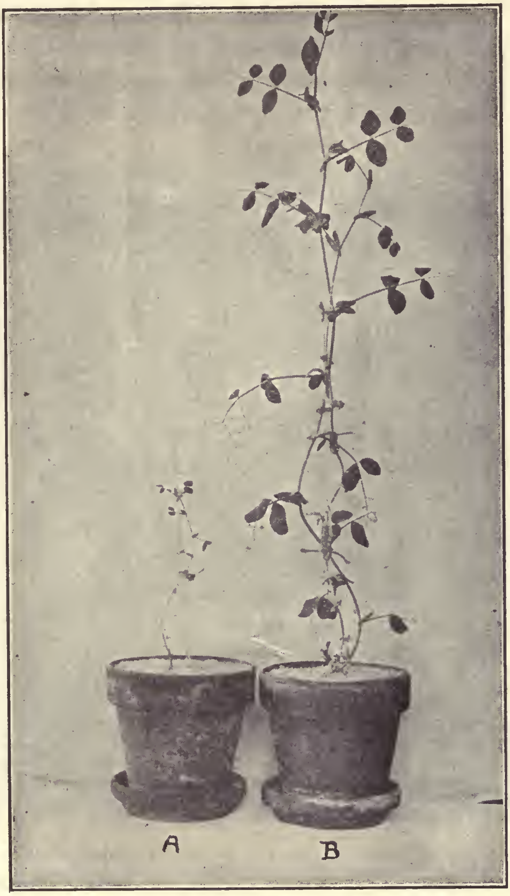


FIG. 135.—These two pea plants were grown in clean quartz sand to which had been added small quantities of all the necessary elements of plant food *except nitrogen.* The conditions were exactly identical except that plant A was without root nodules (see Fig. 136) and plant B had numerous nodules well developed (see Fig. 137). (*Mich. Exp. Station.*)

The top dressing of lawns with lime, marl or wood ashes encourages the appearance of white clover; an adequate supply of lime makes possible the successful growing of alfalfa in almost any soil, while the leguminous vegetation of limestone soils is proverbially vigorous. The favorable influence of lime is due to the direct action on the plants as well as on the bacteria in the soil. Similarly, the tubercle bacteria are favorably affected in their survival and multiplication by an abundant supply of organic matter. On the other hand, acid soils or those deficient in humus and inadequately aerated are but ill suited to the activities of *Ps. radicicola.*

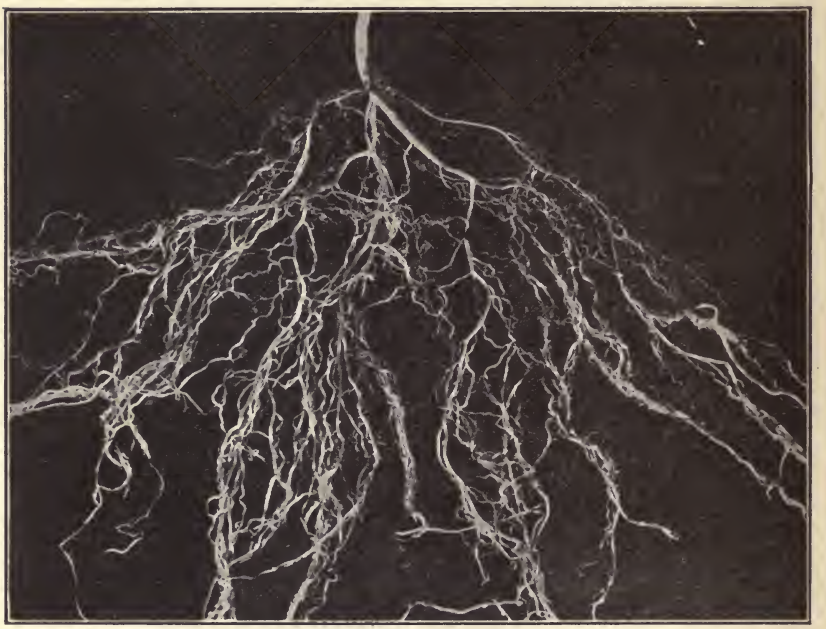


FIG. 136.—Roots of Plant A without nodules (Fig. 135).

SOIL INOCULATION*

By soil inoculation is now understood the adoption of some artificial method for supplying suitable quantities of nitrogen-fixing organisms to soils deficient in these types. The first attempts at soil inoculation were made in 1886 by Hellriegel and Wilfarth during the course of their studies on the cause of nitrogen accumulation by legumes. They found that when leguminous plants were grown in sterile sand, nodules were formed on the roots only after the addition of a small portion of aqueous extract of fertile soil, or an extract of crushed nodules, or in some cases (lupines and seradella) by soil itself from a field on which these crops had been grown. The first successful artificial production of nodules by the aid of pure cultures was made in 1889 by Prazmowski in the course of studies on the method of entrance of the organism to the root hairs of the host plant.

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* Prepared by S. F. Edwards.

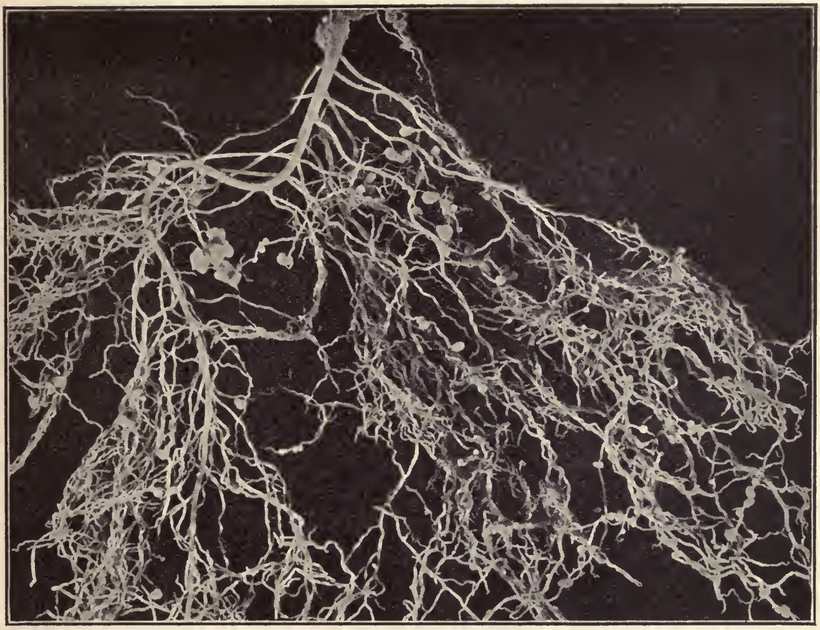

FIG. 137.—Roots of plant B with nodules (Fig. 135).

The first inoculation experiments in a large way were those made in 1887 at the Moor Soil Experiment Station, Bremen, Germany, where earth taken from fields that had borne luxuriant crops of various legumes was scattered over reclaimed heath or swamp soils upon which legumes had not previously grown, with the result that in every instance the yield on the inoculated portions of land was greater than on the uninoculated plots. After such favorable results, it was but a natural step to try the effect of similar applications of soil rich in the nodule-forming bacteria to ordinary cultivated soils of varying character. While results in some cases were eminently satisfactory, in others there was no increase in the vigor or amount of the crop as a result of the inoculation.

METHODS OF SOIL INOCULATION.—From these early experimental results there evolved two general methods of inoculation, namely, the application of soil from an already inoculated field, and the application of pure cultures of the nodule-forming bacteria to the seed before sowing.

Inoculation with Legume-earth.—The use of soil as inoculating material was tried by various experiment stations of the United States, with results not varying widely from those secured in the pioneer experimental work at Bremen. It was found in general that the commonly grown crops, such as the common clovers, peas and beans, made little or no increase as a result of inoculation with old legume-soil. With new crops, however, such as alfalfa and soy beans when they were first introduced, it was found impossible in many places to secure a successful stand until the fields on which these crops were to be grown had received a top-dressing of soil from land that had already grown the crop in question; and it became a common practice to inoculate soil in this manner before seeding with these new crops. It was early observed, however, that this method of soil transfer for inoculation purposes was not an unmixed benefit. Aside from the expense and difficulty of handling and transportation of soil, fungus and bacterial diseases, not only of legumes but of other crops, as well as the seeds of noxious weeds, were transmitted from one field to another and even from one section of country to another. It was to avoid this difficulty that the preparation of pure cultures was introduced.

Inoculation with Pure Cultures. Nitragin.—The first pure culture method was launched in 1896 by Nobbe and Hiltner, German investigators, who prepared cultures of the legume bacteria on nutrient gelatin and arranged with a firm of manufacturing chemists to place them on the market under the trade name of *Nitragin.*

Dried Cultures.—In the United States the matter of pure cultures was first taken up by the Department of Agriculture about 1902. Cultures of the nodule-forming bacteria were cultivated in nitrogen-free culture media, dried on cotton and distributed to farmers with a small package of salts from which a culture solution was to be made by the farmer and applied to the seed. This method gave poor results, chiefly because the bacteria could not withstand the drying on cotton. Afterward the cultures were sent in a liquid condition with somewhat more satisfactory results. The dry cotton cultures were exploited for a time by a commercial firm under the name of *Nitro-culture*, and somewhat similar cultures were placed on the market in England under the name of *Nitro-bacterine*. Cultures of both kinds, however, were shown to be valueless, both by microbiological and by planting tests.

Cultures on Agar.—Very satisfactory results were secured from the use of pure cultures at the Ontario Agricultural College, Guelph, where Harrison and Barlow, in 1905, originated the method of growing the bacteria on a nitrogen-poor agar medium. By this method, the farmer has simply to apply the bacteria to the seed just before sowing. These cultures, used on all the common legumes, sown in all kinds of soil, gave favorable results in 65 per cent of cases in trials extending over a period of ten years. Similar agar cultures are now prepared by commercial firms who have adopted the method of Harrison and Barlow, and also by some of the U. S. Agricultural Experiment Stations.

*Cultures in Soil.**—Temple has suggested that sterilized soil with the addition of a small amount of leguminous material furnished a very good medium for the propagation of legume bacteria and is suitable for their distribution.

Attempts have been made to put on the market cultures containing so-called "fertilizing bacteria" good for "all crops," but the tests made with these cultures have thus far failed to bear out the claims made for them. The successful commercial exploitation of cultures containing strong cellulose and protein decomposing organisms, non-symbiotic nitrogen-fixing organisms, strong nitrifying organisms and other useful bacteria is still to be accomplished.

Importance of Inoculation.—Inoculation with pure cultures affords the farmer a rapid, easy, and cheap method of supplying the bacteria essential for getting a successful stand of any legumes. Failure to secure a benefit from this method of inoculation may usually be attributed to unsuitable soil conditions rather than any inherent failing in the cultures used. No method of inoculation will compensate for poor physical or chemical condition of the soil itself. The principle of using artificial cultures to be applied with the seed is sound, and if the cultures contain large numbers of virile bacteria, there is little reason why they should not prove of benefit when used under soil conditions that would seem to need inoculation.

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* Prepared by Jacob G. Lipman.

Azotobacter Cultures.—Some experimental work has been done in the use of cultures of Azotobacter for soil inoculation. The results are contradictory, and more work needs to be done to prove the value of such cultures.

CHAPTER IV

CHANGES IN INORGANIC CONSTITUENTS

Weathering Process

Origin and Formation of Soil.—Rock surfaces exposed to the action of rain, sunshine and frost lose their fresh appearance, become pitted and uneven, and gradually crumble into larger and smaller fragments. In the course of time the layer of disintegrated material becomes deeper and its constituent particles smaller—thanks to the uninterrupted process of subdivision. Finally, lichens, algæ and bacteria make their appearance, the organic débris accumulates, and higher plants begin to find a suitable environment for their development, The rock has changed into soil.

Influence of Biological Factors.—Soil-formation is not entirely a mechanical or chemical process. Even before the layer of weathered rock acquires any appreciable depth microscopical and macroscopical forms of life gain a foothold on the uneven surface. With the aid of sunlight they build organic compounds and make use of the combined or elementary nitrogen of the atmosphere. Their life activities result in the production of carbon dioxide and of varying organic and inorganic acids which in their turn react with the constituents of the rock particles. In this manner the biological activities become of utmost moment in the transformation and migration of mineral substances in nature. They assume an important rôle in the circulation of calcium and magnesium, with the accompanying phenomena that find most striking expression in the formation of caves and canyons in limestone strata. They assume a no less important rôle in the circulation of sulphur; in the accumulation and removal of available potash compounds in the soil, as well as in the transformation of phosphorus and its migration from inorganic to organic compounds.

Lime and Magnesia

Removal and Regeneration of Carbonates.—Lime and magnesia are present in soils in different combinations. They may occur as silicates, carbonates, phosphates, humates, sulphates, etc. In humid climates the carbonates are being continually removed from weathered rock material, as is plainly shown by the composition of drainage waters. The losses become much greater in cultivated soils—thanks to the humus and the microörganisms present in them. The absolute amounts lost from year to year will depend on the proportion of lime and magnesia in the soil, the mechanical composition of the latter, its content of humus and the methods of tillage and fertilization. According to Hall the soils of the experiment fields at Rothamsted, containing about 3 per cent of calcium carbonate, are losing lime at the rate of 362 kg. to 453 kg. (800 to 1,000 pounds) per acre annually. In certain sections of Scotland where liming has been practised for a long time the farmers estimate the loss of lime from the land at 6 bushels per acre, annually; that is, approximately at the rate of 226 kg. to 272 kg. (500 to 600 pounds). In New Jersey, New York, Pennsylvania and other eastern states farmers who use lime more or less regularly apply 1 ton of it at the beginning of each five-year rotation. This would provide for an annual loss of 181 kg. (400 pounds) per acre. The loss of lime and magnesia is increased under intensive methods of agriculture. When animal manures and green manures are employed, microbial activities are stimulated, the production of carbon dioxide is encouraged and the loss of the soluble calcium bicarbonate made greater. The removal of lime is hastened even to a more striking extent when ammonium salts are applied to the land. The resulting nitrification and loss of lime are illustrated by the following equation:

$$(NH_4)_2SO_4 + 2CaCO_3 + 4O_2 = Ca(NO_3)_2 + CaSO_4 + 4H_2O + 2CO_2$$

As was already indicated, the loss of calcium and magnesium carbonate from the soil is effected largely through the activities of bacteria and of other microörganisms. At the same time microörganic life is responsible for the restoration of varying amounts of carbonates. It has been demonstrated that, in the weathering of the complex silicates, carbonates and silicic acid may be formed in considerable quantities. In the presence of decaying organic matter and the consequent evolution of carbon dioxide the formation of carbonates from silicates may be extensive enough to balance the losses. Similarly, calcium carbonate may be formed in the soil from humates and from the calcium salts of simpler organic acids. They may be formed, also, through the activities of denitrifying and other reducing bacteria from the corresponding nitrates and sulphates. As pointed out by Nadson ammonium carbonate produced in the decomposition of protein compounds may react with calcium sulphate as follows:

$$(NH_4)_2CO_3 + CaSO_4 = CaCO_3 + (NH_4)_2SO_4$$

Moreover, calcium sulphate may be reduced to sulphide and may react with carbon dioxide as follows:

$$CaS + CO_2 + H_2O = CaCO_3 + H_2S$$

Magnesium would be subject to similar reactions and Nadson has observed the formation of a mixture of calcium and magnesium carbonates (corresponding to dolomite in composition) in media inoculated with a pure culture of *B. (Proteus) vulgaris.*

LIME AS A BASE.—The carbon dioxide generated in vast amounts in the life processes of most soil bacteria, the nitrous and nitric acids formed by the nitro-bacteria, the sulphuric acid produced in the oxidation of hydrogen sulphide and of sulphur by the so-called sulphur bacteria, and the great variety of organic acids formed in the decomposition of carbohydrates, fats and proteins all react with basic substances in the soil. Of these basic substances calcium carbonate is by far the most prominent. Combining with the different acids it maintains a favorable reaction for microörganic life in the soil.

The calcium salts thus formed are more or less soluble. In this manner enormous amounts of lime are annually carried to the ocean as bicarbonate, and to an appreciable extent also as nitrate and sulphate. Thus soil bacteria help to furnish shell fish and other forms of marine life, the material necessary for the building of their skeletons. In the course of ages the latter become a portion of the solid land and as coral reefs, chalk cliffs and marl beds offer to microörganisms a new opportunity to start calcium carbonate on its migrations.

EFFECT OF CALCIUM AND MAGNESIUM COMPOUNDS ON BACTERIAL ACTIVITIES.—Being basic in character calcium and magnesium carbonates are of great service in maintaining a suitable reaction in the soil. But somewhat apart from this service calcium and magnesium compounds seem to be particularly important for the growth of certain organisms. It has already been observed by Winogradski and Omelianski that magnesium carbonate is especially useful in facilitating the isolation and culture of nitrate bacteria. Heinze and others have noted the favorable action of calcium carbonate on the growth of *Azotobacter*, while the beneficial influence of calcium carbonate and sulphate on the development of *Ps. radicicola* has been repeatedly observed by different investigators.

Lipman and Burgess found that calcium carbonate stimulates nitrogen fixation by *A. chroöcoccum* in solution, but is without effect in soil. Magnesium carbonate is very toxic both in soil and in solution for cultures of *A. chroöcoccum* even in concentration of 0.1 per cent. Calcium carbonate exercises a protective action against the toxic properties of magnesium carbonate.

PHOSPHORUS

AVAILABILITY OF PHOSPHATES.—Phosphorus exists in the soil largely in the form of phosphates of calcium, magnesium, iron and aluminum. A small portion of it occurs in organic combination in lecithin, phytin and other compounds. The soil phosphates possess a very slight degree of solubility and often fail to become available rapidly enough to meet the demands of the growing crop. Fortunately the presence of carbon dioxide generated from decaying organic matter hastens the solution of the inert phosphates, thus:

$$Ca_3(PO_4)_2 + 2CO_2 + 2H_2O = Ca_2H_2(PO_4)_2 + Ca(HCO_3)_2$$

For this reason a maximum supply of available phosphates may be secured by plants in the presence of readily decomposable organic matter.

Apart from carbon dioxide as a means for making available inert phosphates, bacteria produce organic and inorganic acids that are of direct service. The influence of nitrous, nitric and sulphuric acids, all of them products of bacterial activity, is undoubtedly of some importance. The influence of lactic, acetic and butyric acids, as well as of the more complex humic acids, must be of considerable moment. For instance, in the decomposition of bone meal by *B. mycoides*, Stoklasa found that 23 per cent of the phosphoric acid had become soluble, whereas in similar uninoculated portions of bone meal only 3 per cent of soluble phosphoric acid was found. The significance of organic acids produced by microörganisms is brought out even more strongly in the loss of phosphates from acid soils.

In so far as the organic phosphorus compounds are concerned bacterial activities are important in that the processes of decay restore the phosphorus to circulation. Hence, it will be seen that microörganisms are directly concerned in the migration of phosphorus from the soil to the plant and from the plant back to the soil.

RELATION OF PHOSPHORUS TO DECAY AND NITROGEN FIXATION.—Just as bacteria influence the transformation of phosphorus compounds in the soil, so phosphorus itself affects the growth and activities of bacteria. As one of the essential constituents of living cells it reacts on the growth of microörganisms and influences species relationships. There are undoubtedly species whose phosphorus requirement is greater than that of other species. Indeed, conditions may arise that favor the rapid assimilation of soluble phosphates by bacteria. In that case the microörganisms would act as competitors to the higher plants. Among the species favorably affected by an abundant supply of phosphates *Azotobacter* is quite prominent. Hence nitrogen fixation is in a measure dependent upon a proper supply of phosphorus compounds.

Fred and Hart have shown that the potassium ion does not materially influence ammonification; soluble phosphates cause large increases in the number of bacteria, ammonification and carbon dioxide production. By applying soluble phosphates to the soil crop production is increased, and it is due, in part, to the promotion of bacterial activity. The increased bacterial activity results in a more rapid decomposition of the organic matter, thus making available for the growth of crops larger quantities of nitrogen and probably of minerals.

SULPHUR

SULPHUR COMPOUNDS IN THE SOIL.—Sulphur occurs in the soil in the form of sulphates and in that of organic compounds. In ill-aerated soils the reduction products of sulphates, viz., sulphites, sulphides and even elementary sulphur, may be present in small amounts as a transition stage. According to Berthelot and André the protein compounds of the soil humus are quantitatively more important than the sulphates. However, this is not true of arid and semi-arid soils in which sulphates represent a larger store of combined sulphur than is contained in organic substances.

Sulphur-phosphate Composts.—In the composting of sulphur, ground rock phosphate (floats) and soil certain soil bacteria oxidize the sulphur into sulphuric acid, which acts upon the insoluble rock phosphate and makes it soluble and available for higher plants. The best combination found at the New Jersey Agricultural Experiment Station consists of 100 parts of soil, 120 parts of sulphur and 400 parts of rock phosphate, inoculated with material from an old compost. The bacteria causing the oxidation of sulphur were isolated at the New Jersey Agricultural Experiment Station and were found to be short, non-motile, Gram-positive rods. They are obligate aerobes and are able to convert sulphur into sulphuric acid.

Sulphur Bacteria.—In the decomposition of protein compounds with a limited supply of air, hydrogen sulphide and mercaptans are evolved. The quantities of hydrogen sulphide produced may be large enough to become perceptible to the sense of smell, as happens in the putrefaction of eggs. At the bottom of seas, rivers, lakes and ponds (in canals, ditches, swamps, etc.) as well as in finer-grained soils the production of hydrogen sulphide goes on almost uninterruptedly owing to the activities of a great variety of bacteria. The hydrogen sulphide thus generated serves as a source of energy to a group of organisms known as sulphur bacteria. The oxidation of the hydrogen sulphide by these bacteria may be expressed by the following equations:

$$2H_2S + O_2 = 2H_2O + S_2$$
$$S_2 + 2O_2 = 2SO_2$$

The sulphur dioxide produced is further changed into sulphuric acid in the presence of oxygen and water. In its turn the acid reacts with some base, usually calcium carbonate, resulting in the formation of calcium sulphate. Thus:

$$SO_2 + O + H_2O = H_2SO_4$$
$$H_2SO_4 + CaCO_3 = CaSO_4 + H_2O + CO_2$$

We owe much of our knowledge concerning the sulphur bacteria to Winogradski. This investigator showed that in places where hydrogen sulphide is generated in considerable quantities sulphur bacteria grow vigorously and accumulate granules of sulphur within their cells. When the cells containing sulphur granules are removed to suitable media, in which no hydrogen sulphide is present, the sulphur seems to be gradually oxidized and disappears and the bacteria finally die of starvation. Thanks to the sulphur bacteria, the higher plants are enabled to utilize again the sulphur once locked up in plant and animal tissues, and liberated thence by decay bacteria. The circulation of sulphur is thus made possible and the cycle is completed when the sulphates are again used by plants to build protein compounds. It may also be noted in this connection that "*Thiobacillus denitrificans*," described by Beyerinck, may also oxidize elementary sulphur. In this case, however, the oxygen is derived from nitrates instead of the atmosphere. Thus:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

SULPHOFICATION.—Lint has found that under optimum temperature and moisture conditions, sulphur applied at the rate of 600 pounds per acre was almost completely oxidized within ten weeks. Boullanger and Dugardin in explaining the fertilizing action of sulphur on the basis of its effect on the supply of available nitrogen found that ammonification was increased by small amounts of sulphur, nitrogen-fixation was not affected and nitrification was depressed. It has been pointed out by Kossovitch, Brioux and Puerbet that the mechanism of sulphur fertilization is very complex and that the oxidation of free sulphur occurs entirely by bacterial and not by chemical means. Brown and Kellogg have recently advanced evidence to prove that soils have a definite sulphofying power which is determinable in the laboratory by a newly devised method. They claim that the process of sulphofication is mainly brought about by bacterial action, but probably there is also a small production of sulphates in soils due to chemical action.

It has been observed that soils differentiated by various treatments, vary widely in sulphofying power, the presence of organic matter being responsible for an increase up to a certain point. Aeration and moisture must be optimum for favorable sulphofication while the addition of carbohydrates to soils depresses the process.

SULPHATE REDUCTION.—The fact that sulphates may be reduced to sulphides in the presence of organic matter has been known for many years. In compost heaps, and at the bottom of seas, lakes and rivers, the reduction of calcium sulphate is of common occurrence. Similarly, ferrous sulphate may be reduced in water-logged soils and in swamps and may give rise to deposits of bog iron. But while sulphate reduction is of common occurrence in certain localities, it has been shown by Beyerinck and also by van Delden, that the reduction can be accomplished in artificial media by specific microörganisms. Two species isolated by these investigators have been named *Sp. desulphuricans* and *Msp. æstuarii.* When grown under anaerobic conditions in culture media supplied with combined nitrogen and organic nutrients these organisms were found capable of reducing sulphates. The oxygen withdrawn from the sulphates was used for the oxidation of organic matter in a manner analogous to that in nitrate reduction where the oxygen is derived from the nitrates. Apart from the two organisms that cause the specific reactions just noted, there are many common soil bacteria that may be responsible for sulphate reduction in a less direct manner. Nadson has observed that when the supply of oxygen is limited calcium sulphate may be reduced to sulphide by *B. mycoides* and by *B.* (*Proteus*) *vulgaris.* The calcium sulphide according to him may react with carbon dioxide and water, giving rise to the formation of hydrogen sulphide. Thus:

$$CaS + CO_2 + H_2O = CaCO_3 + H_2S$$

The hydrogen sulphide derived from sulphates or from proteins becomes a source of energy to the sulphur bacteria as already noted in the preceding pages.

POTASSIUM

THE TRANSFORMATION OF POTASSIUM COMPOUNDS IN THE SOIL.—Potassium occurs in the soil largely in the form of silicate minerals. Smaller amounts occur as nitrate, carbonate and in organic compounds. The portion present as silicates is often very large in clay-loam soils, amounting not infrequently to 22,679 kg. to 34,019 kg. (50,000 to 75,000 pounds) per acre-foot. Unfortunately for the farmer, the growing crops fail, in many cases, to secure sufficient quantities of available potash for their rapid development, notwithstanding these enormous stores of potassium compounds. However, when sufficient quantities of readily fermentable organic matter are present and the generation of carbon dioxide is rapid the silicates weather sufficiently fast to meet the demands of maximum harvests. The part played by carbon dioxide in the transformation of inert potash compounds may be illustrated by the following reaction:

$$Al_2O_3K_2O\,6SiO_2 + CO_2 + 2H_2O = Al_2O_3\,2SiO_2\,2H_2O + K_2CO_3 + 4SiO_2$$

Under actual conditions it is the aim of the farmer to stimulate bacterial activities (and, therefore, the production of carbon dioxide) in his land by the use of animal manures or green manures and of commercial fertilizers. Apart from the influence of carbon dioxide available potash compounds may likewise be formed on account of nitric, sulphuric, acetic, lactic, butyric and other acids produced by different soil bacteria.

OTHER MINERAL CONSTITUENTS

IRON.—The investigations of Ehrenberg, Winogradski, Molisch, Adler, Ellis and others have accumulated a mass of data relating to the so-called iron bacteria. These organisms belong to the class of higher bacteria and recently forms, such as rod-shaped bacteria, have been isolated which have a marked ability to precipitate iron oxide out of solutions of iron salts. Winogradski believed that the reaction is a physiological one in that the microörganisms oxidize ferrous to ferric compounds, and utilize for their growth the energy thus made available. The investigations of Molisch, Adler and Ellis show, however, that the iron bacteria can exist very well without iron compounds and that the precipitation of iron oxide is due to mechanical rather than chemical influences. But whether physiological or mechanical the influence of these microörganisms is felt in the formation of bog iron, and in the filling up of iron pipes; in the latter instance much annoyance is occasionally experienced by those in charge of municipal water supplies.

Compounds of iron are of considerable significance in the life processes of many bacterial species. For instance, it was shown by Lipman and after him by Koch, that *Azotobacter* will not develop in culture media devoid of iron compounds. In field practice small applications of ferrous sulphate often seem to exert a favorable effect on crop growth, and there is reason to suspect that soil-microbial activities are of some moment in bringing about the results noted.

ALUMINUM, MANGANESE, COPPER.—Weathering processes and the relation of carbon dioxide to these processes have already been discussed in connection with calcium and potassium compounds. To a great extent aluminum is affected by these reactions, for in the decomposition of feldspar, kaolinite is one of the important products formed. Hence, bacteria become a factor of considerable importance in the formation of hydrated silicates of aluminum, at least, in the presence of organic matter. Moreover, it is recognized in the ceramic industries that after it is dug clay must undergo ripening in order to be suitable for certain purposes. The ripening process involves the activities of bacteria. Unfortunately very little is known about the reactions that occur in the ripening of clay.

As to manganese and copper there is scarcely any experimental evidence available as to the part played by their compounds in the soil, particularly in so far as they affect microörganic life. To some extent, it is known that where Bordeaux mixture has been employed for spraying potatoes, cranberries, fruit trees, etc., plant growth is subsequently stimulated to a striking extent. In view of the very slight quantities of copper that are actually added to the soil by these sprays, it is possible that the effects noted are caused by stimulated or changed microbial activities. This view finds some support in the influence exerted by copper sulphate on the growth of algæ in lakes, ponds, and shallow streams.

It has also been reported that the decomposition of complex silicates has been effected from powdered minerals by nitrite bacteria.

Antagonism

A subject which bids fair to become a fertile source of investigation is the application of certain biochemical laws, as established by Loeb and Osterhout in the animal and plant worlds respectively, to the effect of salts on the physiological efficiency of soil bacteria in pure and mixed cultures, as well as in the soil. C. B. Lipman has advanced information concerning the antagonism between anions as related to nitrogen transformations in soils, with special reference to the reclamation of alkali lands. Antagonism exists to a more or less marked extent between anions of alkali salts (as for example between $NaCl$ and Na_2SO_4, Na_2CO_3 and Na_2SO_4 and between $NaCl$ and Na_2CO_3) when the ammonifying or nitrifying powers of the soil are employed as criteria. The nitrogen-fixing flora, however, is not similarly affected, apparently offering greater resistance. The practical suggestion carried out of such data then, involves the addition of salts to the toxic salts already contained in a given soil, and thereby improving its ammonifying and nitrifying power.

Variability in Soil Fertility Investigations

Waynick has pointed out that the variations between different soil samples taken from a small area may be of such magnitudes as to throw doubt upon the validity of the experimental data obtained with one or a limited number of samples. A single sample of any soil is of little value as regards determinations which may be made upon it. A composite may be considered of value only after the probable error to which it is subject is known and this can only be determined by the use of a large number of individual samples.

DIVISION IV

MICROBIOLOGY OF MILK AND MILK PRODUCTS

CHAPTER I*

THE RELATION OF MICROORGANISMS TO MILK

CHARACTER OF MILK

The ideal milk is that which reaches the consumer in as nearly as possible the condition in which it leaves the udder of the healthy cow.

The factors which determine the quality of commercial milk may be stated as follows: (*a*) Food value, (*b*) flavor and odor, (*c*) keeping quality, (*d*) cleanliness, (*e*) healthfulness. With the exception of the first, all of these qualities are in part or wholly dependent upon the microbial content of the milk.

Fresh normal milk has a pleasant taste and aroma and is generally liked as a food or drink; but unless properly cared for will not long remain in its normal condition. No article of human diet is more susceptible to undesirable changes, due to the delicate nature ot the milk itself and to the conditions naturally surrounding its production and handling. The injurious changes which commonly occur in milk are of two kinds.

ABSORBED TAINTS AND ODORS

Milk is very quickly affected by odors of any sort. The foreign odor may be absorbed before the milk leaves the udder if the cow has eaten strong feeds, such as cabbage, onions, etc., or it may be absorbed after the milk is drawn from the cow. If milk is exposed to any strong odor, such as silage or foul air, resulting from lack of ventilation in the stable at milking time, these odors will be taken up by the milk with surprising rapidity. If placed in an ice chest with fresh strawberries or pineapple, or foods like cabbage or turnips, the milk will very quickly absorb the odor of these foods. The absorption of any foreign odor gives to milk a decidedly disagreeable taste. This is true even when the odor which is absorbed is pleasant in itself as in the case of strawberries or pineapples. When the "off" flavors are due to absorption they are strongest at the outset and become less pronounced as the milk becomes older, especially if it is subjected to some method of aeration.

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* **Prepared by W. A. Stocking with the exception of the paragraphs treating the acid-forming bacteria, prepared by E. G. Hastings.**

CHANGES DUE TO MICROÖRGANISMS

While absorption of foreign odors is not uncommon, probably most of the undesirable flavors, found in milk when it reaches the consumer, are caused not by absorption but by the growth of microörganisms in the milk. In this class the changes are slight at first and increase with the age of the milk. Changes of this sort include the common phenomena of souring and curdling, the so-called sweet curdling, ropy or slimy milk, bitter flavors, gassy milk and a large variety of changes usually known as barny or cowy odors and flavors. If milk could be kept free from microörganisms, it might be kept for some time without showing perceptible changes in appearance or taste. No other food product will undergo fermentation changes as rapidly as milk because it is an ideal culture medium for the growth of most kinds of microörganisms, especially bacteria and yeasts. Not only does milk contain the needed food elements but, being in liquid form, they are easily available for the use of microorganisms. The proteins and milk sugar are most easily attacked and it is the breaking down of these which causes most of the changes in the milk.

MICROBIAL CONTENT OF MILK

The amount of care exercised in the production and handling is a most important factor in determining the bacterial contamination of milk. On this basis milk may be roughly divided into three classes.

COMMON MILK.—When we recognize the extreme ease with which milk undergoes bacterial changes, we are not surprised to find that ordinary milk, when delivered to the consumer, contains relatively large numbers of bacteria. Age is one of the chief factors in determining the germ content of milk. We, therefore, expect to find the milk in large cities having a much higher germ content than in smaller cities and towns. The normal germ content of ordinary milk as it is found in the cities may be shown by the following tables.

BACTERIA IN BOSTON MILK*

Average taken from 2,394 Samples
From June to September

	Per cent
Below 100,000 bacteria per c.c.	42.0
Between 100,000 and 500,000 per c.c.	29.75
Between 500,000 and 1,000,000 per c.c.	9.75
Between 1,000,000 and 5,000,000 per c.c.	12.75
Above 5,000,000 per c.c.	5.0
Uncountable plates.	0.75

BACTERIAL COUNTS OF CHICAGO (RAW) MILK†

Date	Number of samples	Average count	Lowest count	Highest count
January	64	1,067,000	27,000	5,500,000
April	43	5,948,000	14,000	150,000,000
July	183	12,548,000	8,000	190,000,000

BACTERIA IN MILK OF CONNECTICUT CITIES‡

Bacterial count	Number of samples
Under 50,000	1,707
50,000–100,000	130
100,000–500,000	459
500,000–1,000,000	98
Over 1,000,000	73

These figures give the results of 2,467 samples collected in seventy-five different towns in the State covering a period of one entire year.

Goler gives the average bacterial count for 1,057 samples of market milk collected in Rochester during the year 1909 as 446,099 per c.c. Of these samples 1.79 per cent were above 5,000,000 and 38.4 per cent below 100,000.

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* Data given by Hill and Slack.

† Data given by Tonney.

‡ Data given by Conn.

In Montclair, N. J., the average bacterial count for the year 1918, for the fifteen producers who delivered raw milk, was as follows:

BACTERIAL COUNTS OF RAW MILK, MONTCLAIR, N. J., 1918

Producer's No.	Average Count
1	6,000
2	10,500
3	20,000
4	37,000
5	45,300
6	47,000
7	53,000
8	65,500
9	68,000
10	75,000
11	82,000
12	82,000
13	90,000
14	171,000
15	226,000
Average	71,886

In Ithaca, N. Y., samples taken for the year 1919 gave average bacterial counts by months as follows:

BACTERIAL COUNTS OF MILK IN ITHACA, N. Y., 1919

Month	Average Count
January	111,450
February	145,990
March	101,050
April	93,460
May	123,320
June	115,865
July	66,525
August	47,620
September	151,260
October	11,030
November	27,120
December	91,700

The immense numbers of bacteria found in milk in the large cities are usually the result of the rapid growth of the *Bact. lactis acidi* group resulting from the age of the milk and the temperature at which it has been kept. Such milk may also contain large numbers of those saprophytic organisms which occur freely in nature and which may be abundant about the stables and milk-house. The number of this group depends largely upon the sanitary conditions of production and the initial contamination. In ordinary milk organisms of the *Bact. lactis acidi* type will constitute a very large percentage of those present when the milk reaches the city even before it shows any perceptible signs of souring. During the past few years great progress has been made in the production of clean milk, and at present quite an important part of the general raw milk supply of our cities has a very much lower germ content than it had a few years ago.

Special Milks.—In this class may be considered those milks known as *Selected*, *Inspected*, or *Guaranteed*. As commonly used these terms mean milk which has been produced and handled with considerably more care than ordinary market milk but not with the extreme care required for *certified* milk. While these and similar terms do not always mean milk of the same grade in different places, they usually mean milk produced by herds which have been shown by the tuberculin test to be free from tuberculosis. Considerable care is exercised in all the operations of handling the milk. The result is that these milks usually have a much lower germ content than the ordinary milk supply of the same city. Sometimes the germ content of such milk compares favorably with that of certified milk. These milks may contain various types of normal milk organisms but they should not contain any tubercle bacteria.

Certified Milk.—Certified milk means milk which has been produced according to the regulations of and under the supervision of a medical milk commission. The stables and cows are kept extremely clean. No dust is allowed in the stable at milking time. The cow's flanks and udder are washed just before milking, the milkers wear white suits and wash their hands before milking each cow. Small-top pails are used and the milk is cooled as soon as drawn from the cow. The extreme care exercised in the production and handling of this milk has a very marked effect on the number of bacteria found in it. The following counts are typical of certified milk.

BACTERIAL COUNTS OF CERTIFIED MILK IN DIFFERENT CITIES

Boston, Oct. 1, 1909 to Sept. 30, 1910*

Farm number	Number samples	Average bacteria count
1	17	5,794
2	13	4,176
3	30	6,825
4	12	1,475
5	7	2,294

New York City, Oct., 1909 to Sept., 1910†

Farm number	Average bacteria count
1	11,132
2	10,516
3	8,504
4	16,193
5	2,863
6	11,246
7	23,705
8	5,370
9	15,062
10	459

Chicago‡

Farm number	Number samples	Average bacteria count
1	51	5,612
2	60	4,078
3	43	6,502
4	17	2,553

Brooklyn

Moak gives the average of 321 counts for certified milk delivered in Brooklyn during the first six months of 1910 as 4,095 bacteria per c.c. The best average from any one farm was 561 bacteria per c.c.

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* Data given by Arms.

† Data given by Park.

‡ Data by Heinemann.

SOURCES OF MICROÖRGANISMS IN MILK

The sources from which bacteria get into the milk have been the subject of much investigation during the past few years, until now the chief sources of contamination are pretty well understood. These sources may be grouped in a general way under the following heads:

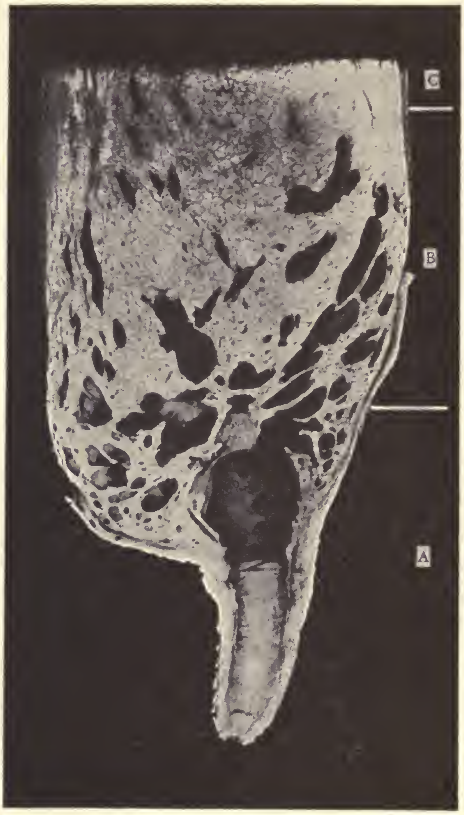

FIG. 138.—Vertical section of one quarter of udder showing teat, milk cistern, and larger milk ducts. (*After Ward and Hopkins.*)

INTERIOR OF THE COW'S UDDER. *Healthy Udders.*—Milk as it is secreted by the normal udder of a healthy cow is probably free from bacteria. It is very difficult, however, to obtain milk from the udder which does not contain bacteria in greater or less numbers. This is due to the fact that immediately after secretion the milk becomes contaminated by bacteria which exist in the interior of the udder. Early investigators, notably de Freudenreich and Grotenfelt, believed that milk while in the udder was entirely free from microörganisms. Later investigations, however, by Moore, Ward, Bolley, Hall and others, have shown that the healthy udder normally contains bacteria in appreciable numbers. It has been found that bacteria are present even in the upper portions of the udder in the small milk passages leading from the secreting cells. These organisms, which normally exist in the milk passages of the udder, gain entrance through the orifice in the end of the teat where they find suitable conditions for growth and, once inside, work up through the milk cistern to the larger milk ducts and finally though all parts of the udder (Fig. 138). The number of bacteria found in the udder varies widely in different cows as may be seen by the following figures:

BACTERIAL CONTENT OF ENTIRE MILK OF DIFFERENT COWS

Cow No. 1	850 bacteria per c.c.
Cow No. 2	750 bacteria per c.c.
Cow No. 3	25 bacteria per c.c.
Cow No. 4	112 bacteria per c.c.
Cow No. 5	70 bacteria per c.c.
Cow No. 6	1,850 bacteria per c.c.

If portions of milk are taken at different intervals during the process of milking in such a way that all external contamination is prevented, it will be found that the first few streams of "fore-milk" contain many more organisms than the milk drawn later. After the first ten or twelve streams the number of organisms will decrease quite rapidly, normally becoming less and less until the final strippings, when there is usually a marked increase. This condition indicates that the larger number of organisms exist in the milk cistern and larger milk ducts in the lower part of the udder and are therefore removed during the early part of the milking. The increase at the end of the milking is probably due to the greater manipulation, resulting in dislodging some of the organisms which have adhered to the walls of the milk passages.

Not only does the number of organisms in different cows vary, but there is a marked difference in the different quarters of the same udder, as shown by the following figures.

BACTERIA IN DIFFERENT QUARTERS OF COW'S UDDER[*]

	Right front quarter of udder		Left front quarter of udder		Right back quarter of udder		Left back quarter of udder	
	No. samples	Average per c.c.	No. samples	Average per c.c.	No. samples	Average per c.c.	No. samples	Average per c.c.
Herd of 1900–02	79	419	77	378	80	653	80	617
Herd of 1910–11	185	199	174	130	185	636	186	698
Herd of A. G. L.	46	161	46	107	46	597	46	342
Averages	......	249	......	191	......	635	......	625

Average germ content per c.c. in 316 samples from herd of 1900–02	518
Average germ content per c.c. in 730 samples from herd of 1910–11	420
Average germ content per c.c. in 184 samples from herd of A. G. L.	320
Average germ content per c.c. in 1,230 samples from 78 cows	428

The number of organisms normally found in the udder is much smaller than would be expected when we consider the fact that ideal conditions of food and temperature are provided there for bacterial growth. The relatively small number of organisms is perhaps due to some germicidal action existing in the udder. Attempts to increase the germ content in the udder by injecting cultures of different species of saprophytic bacteria have failed to produce a continued increase, the injected organisms usually decreasing very rapidly in numbers until they disappear at the end of a few days. From the standpoint of ordinary market milk, the number of bacteria found in the healthy udder is so small that it is of little commercial importance. In dairies where a very small germ content is desired, however, this source of infection must be taken into account and in certain cases individual cows, which normally have a high bacteria content in the udder, can be discarded to advantage.

It is evident that many species do not find the conditions in the udder suitable for their growth, since investigations have shown that comparatively few species exist for any length of time in the healthy udder. Certain types of micrococci are the predominating forms with occasional cultures of other species. The *Bact. lactis acidi* type does not thrive in the udder. The types of organisms commonly found there do not seem to develop rapidly in the milk when it is held at low temperatures and fail to produce any appreciable changes in it during the normal life of market milk.

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[*] Harding and Wilson: Technical Bul. No. 27, N. Y. Agric. Exp. Sta., 1913.

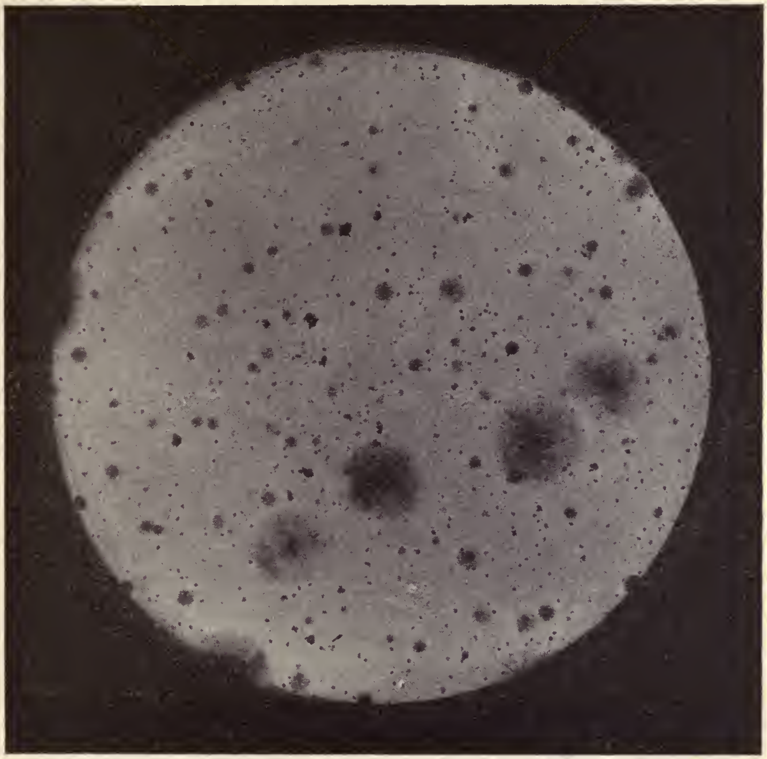

FIG. 139.—Colonies developing in agar plate held for ten seconds in position of milk pail after udder was brushed gently with the hand.

Diseased Udders.—If, however, the cow is suffering from disease in the udder, the bacterial condition may be quite different from that described above. In this case, the milk may be filled with the specific bacteria before it leaves the udder. In cases of inflammatory trouble or tuberculosis in the udder the milk may contain very large numbers of organisms, frequently many millions per c.c. at the time the milk is drawn.

EXTERIOR OF COW'S BODY.—The nature of the cow's coat and the condition under which she is normally kept favor the accumulation of dust and bacteria upon her body. Unless special care is taken to keep the cow's body free from dirt, the organisms which fall into the milk from this source at milking time will constitute one of the most important sources of contamination. The importance of this source of contamination may be recognized when we see what large numbers of microörganisms may be carried by small particles of dust or an individual cow hair. The amount of this source of contamination is indicated by the marked reduction in germ content resulting from the use of a small top pail (page 442).

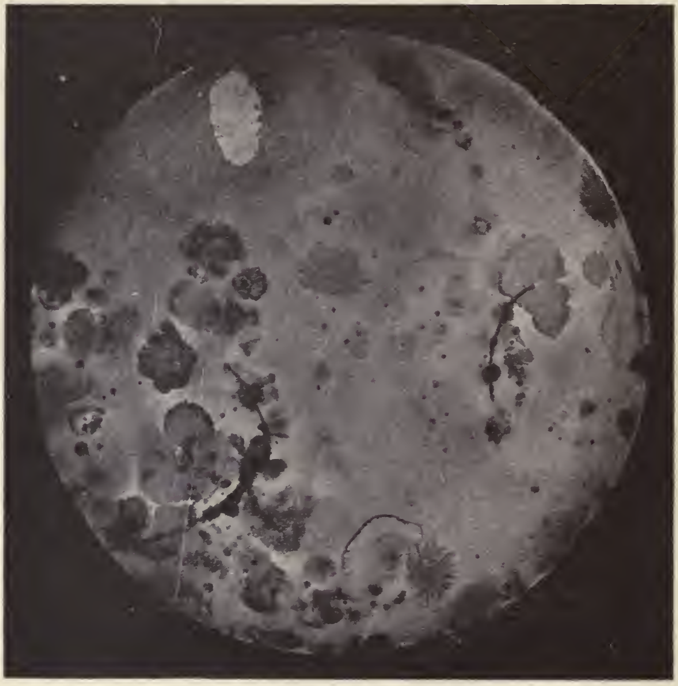

FIG. 140.—Colonies developing from cow-hairs planted in agar plate.

The importance of this source of contamination depends very largely upon the conditions under which the cows are kept and the care exercised in cleaning just previous to milking. In many of the certified milk dairies this source of contamination is reduced to a minimum and has little effect upon the milk.

ATMOSPHERE OF STABLE AND MILK HOUSE.—The atmosphere of the stable may be an important factor in influencing the bacterial content of fresh milk In well kept stables fairly free from dust this source of contamination is usually not important but in stables where the air is full of dust at time of milking, the germ content of the milk may be appreciably increased from this source. In sanitary dairies this factor is fully recognized and every effort is made to prevent the presence of dust in the atmosphere at the time of milking

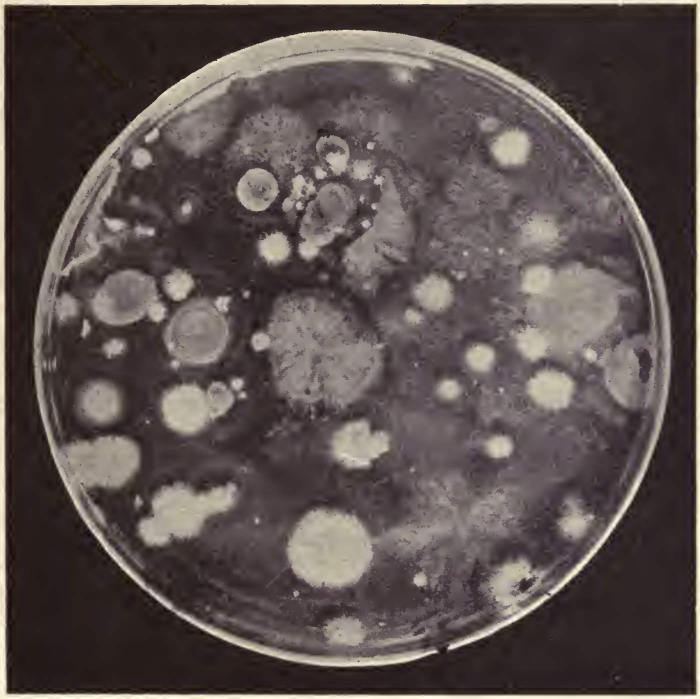

FIG. 141.—Colonies developed from a bit of dust found in cow stable. Agar plate culture.

THE MILKER.—Not infrequently the milker himself is a source of contamination. If his clothing and hands are dirty or if he brushes against the cow, the dust thus dislodged may carry into the milk large numbers of microörganisms. This is shown in the difference in the germ content of milk drawn by two men milking in the same barn under identical conditions.

DIFFERENCE IN NUMBER OF BACTERIA IN MILK DRAWN BY MEN IN SAME STABLE

	Number of milkings	Number of bacteria per c.c.
Milker No. 1	19	2,450
Milker No. 2	19	17,100

THE UTENSILS.—If properly cared for, the dairy utensils should not add to the germ content of the milk. Not infrequently, however, they are faulty in construction. In open seams and other places the milk may accumulate and not be thoroughly washed out. Usually when utensils of this sort are used, the methods for washing and sterilizing are not sufficient and bacteria multiply in large numbers in the cracks and crevices and contaminate each new lot of milk put into them. Sometimes the utensils which are properly constructed may contaminate the milk because they have not been properly cleansed and sterilized. The possible effect of the utensils on the germ content of the milk put into them is shown by recent work done at the Illinois Agricultural Experiment Station.* It was found that when the utensils were properly washed and thoroughly steamed and dried they did not add many bacteria to the milk. On the other hand, when they were not well steamed and especially when allowed to stand wet for several hours they added very large numbers of bacteria to the milk. This is shown by the following table.

AVERAGE NUMBER OF BACTERIA ADDED TO FIFTY LITERS OF MILK BY THE VARIOUS UNSTEAMED UTENSILS IN WHICH IT WAS HANDLED

Source of bacteria	Number of bacteria per cc. of milk	Total number of bacteria
Sources other than utensils	5,000	250,000,000
3 pails	54,635	2,731,750,000
1 strainer	7,315	365,750,000
1 clarifier tank	8,038	401,900,000
1 clarifier	141,340	7,067,000,000
1 cooler	50,900	2,545,000,000
1 bottle-filler tank	83,246	4,162,300,000
Total	350,000	17,523,700,000
Total for utensils	345,000	17,273,700,000

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* Illinois Bull. 204, 1918.

These figures indicate that the utensils may play a much more important part in determining the total germ content of milk than was formerly supposed. The use of steam is the most efficient means of sterilizing all dairy utensils, but boiling water may give very satisfactory results if used at actual boiling temperature. If not used at the boiling temperature some of the resistant organisms will not be killed and will be left to inoculate the fresh milk. The ropy milk organism, *B. lactis viscosus*, often remains in the utensils from day to day in this way.

WATER SUPPLY.—Sometimes the water used for washing the dairy utensils is a serious source of contamination. Serious epidemics of disease have been traced to this source where the utensils were washed with water contaminated by typhoid or other disease organisms and were not sufficiently sterilized to kill those remaining in the utensils. Such dairy troubles as ropy milk and gassy milk may be caused by the water used for washing purposes.

METHODS OF PREVENTING CONTAMINATION OF MILK

INDIVIDUAL COWS.—Normally the number of microörganisms found in the udder is not sufficient to be a serious source of contamination for market milk. There are, however, certain cows which have a much higher germ content than others, and where a very low count is desired in the milk, it may sometimes be advisable to eliminate such cows from the herd.

CARE OF THE COW'S BODY.—In order to reduce to the minimum the contamination from the cow's body, she should be kept as clean as possible. Dust should not be allowed to accumulate in her coat. It is well to keep the hair of the flank and udder clipped in order to prevent the accumulation of dust and also to facilitate the process of cleaning. The use of a damp cloth for wiping the flank and udder at milking time is a very efficient means of reducing this source of contamination. The beneficial effect of this method may be seen in the following table.

Even when considerable care is taken to clean the surface of the cow's body, there will still be some organisms which may fall into the pail at milking time. This number can be very materially lessened

EFFECT OF WIPING UDDER AND FLANK WITH A DAMP CLOTH AS SHOWN BY BACTERIAL COUNTS OF MILK

Number of experiments	Date	Treatment	Bacteria per c c.
1	Apr. 13	Not wiped	2,780
		Wiped	530
2	Apr. 15	Not wiped	1,310
		Wiped	310
3	Apr. 16	Not wiped	800
		Wiped	754
4	May 28	Not wiped	1,130
		Wiped	590

by reducing as far as possible the area through which dust can fall into the milk pail. This can be accomplished by the use of a milking pail with a small top.

VALUE OF SMALL TOP PAIL IN REDUCING GERM CONTENT OF MILK

Experiment	Kind of pail	Bacteria per c.c. of milk
No. 1	Open	15,500
	Small top	7,750
No. 2	Open	3,700
	Small top	1,100
No. 3	Open	30,000
	Small top	4,700

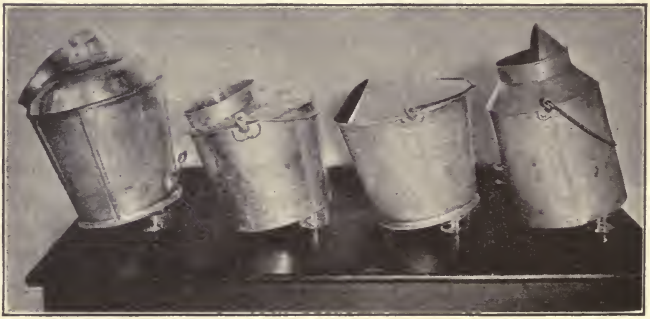

FIG. 142.—Some different styles of small top milking pails which are practical and efficient.

Results of extended trials in different barns demonstrate the fact that approximately two-thirds of the organisms which would fall into an ordinary open pail are kept out by the use of a pail of the type shown in No. 3, figure 142. The following figures give average results of trials in three different barns.

BACTERIAL COUNTS OBTAINED WITH OPEN AND SMALL TOP PAILS

Barn	Kind of pail	Average bacterial count
No. 1	Open	1,610
	Covered	280
No. 2	Open	6,000
	Covered	3,000
No. 3	Open	33,000
	Covered	1,740

AVOID DUST IN THE ATMOSPHERE.—Many of the necessary operations of the cow stable stir up large quantities of dust and fill the air with microörganisms. It is astonishing to see how many bacteria can adhere to a small piece of hay or may be found in a gram of some of our common dairy feeds. When these materials are fed dry just previous to milking time, the atmosphere of the stable will be filled with organisms some of which may settle into the milk while it is exposed during the process of milking. The effect of this source of contamination in one stable may be seen by the following experiments:

BACTERIAL CONTENT OF MILK AS AFFECTED BY FEEDING DRY HAY AND GRAIN

Experiment	Date	Nature of sample	Number bacteria per c.c.
No. 1	May 4	Before feeding	350
		After feeding	1,450
No. 2	May 17	Before feeding	2,900
		After feeding	4,400
No. 3	May 18	Before feeding	4,100
		After feeding	7,200

In another stable* where the sanitary conditions were above the average and where all the conditions were carefully controlled, the atmosphere added from 7 to 937 germs to each c.c. of the milk, the number varying with the amount of dust in the air.

* N. Y. (Geneva) Agr. Exp. Sta. Bull. 409.

DAIRY UTENSILS.—All utensils which are to be used in connection with milk should be so constructed that there are no cracks or crevices in which the milk can accumulate and from which it is not easily washed. A milk pail with an open seam may be the cause of serious trouble in the dairy. The dairy utensils should be simple in construction, and so made that they can be thoroughly cleansed with ease and made of such material that they can be thoroughly sterilized either with water which is actually boiling or in steam. They should then be thoroughly dried and kept so till again needed for use. When moisture is left in cans and other utensils bacteria can grow rapidly and be the means of serious contamination when fresh milk is poured into them.

THE MILKER.—No food material requires greater care and cleanliness on the part of those handling it than does milk. All persons having to do with the handling of this delicate food product should constantly keep in mind that clean hands and clothing and extreme cleanliness in every operation is very necessary if milk of good quality is to be obtained.

GROUPS OR TYPES OF MICROÖRGANISMS FOUND IN MILK AND THEIR SOURCES

In studying the types of bacteria found in milk, it is convenient to arrange them in groups based upon their action on the milk and their effect upon persons consuming it. There are certain types of organisms which are very troublesome to the milk dealer but which are not injurious to the consumer. Other species which may be of little or no significance from their action on the milk are of greatest significance from the standpoint of the consumer since most of the disease organisms which may be carried by milk have no appreciable action upon it. Still other forms are of but little importance to either the dealer or the consumer and others are troublesome to both.

GENERAL SIGNIFICANCE OF ACID-FORMING BACTERIA.—Of all the bacteria that find their way into milk, those that are able to ferment the milk sugar, producing from it different kinds and amounts of acids, find more favorable conditions for growth at ordinary temperatures, 15° to 45°, than do those belonging to other groups. Because of their greater rapidity of growth and because of the inhibiting effect of their by-products upon the other groups of bacteria, the acid-forming types tend to predominate in milk and the specific change which they produce, the souring, is of such common occurrence that it is often looked upon as something inherent in milk.

Groups of Acid-forming Bacteria.*—The acid-forming bacteria that are constantly present in milk represent many kinds which differ in morphology, in cultural characteristics, and in their products of fermentation. They may be divided into four groups that vary greatly as far as their importance in the handling of milk is concerned. If milk is produced under clean conditions and is kept at temperatures ranging from 15° to 35°, the acid fermentation will be almost wholly due to a group of bacteria closely allied to one of the pathogenic forms, *Strept. pyogenes* (Rosenbach). To representatives of this group, which is of the greatest importance in all phases of dairying, have been given various names by different investigators. The most important organism of this group is one to which the name *Bact. lactis acidi* is applied. The group undoubtedly includes a large number of organisms, all of which produce, however, a similar change in milk.

Second in importance is a group of organisms, of which the best known representatives are *B. coli communis* and *Bact. lactis aerogenes*. A large number of organisms of this group have been described and named. The most important characteristics of the representatives mentioned will, however, suffice to characterize the group. A third group is represented by *Bact. bulgaricum* and the rod-shaped organisms that were first studied in detail by de Freudenreich. A fourth group includes many acid-forming cocci, some of which exhibit proteolytic properties while others do not. Organisms of the third and fourth groups exert little or no effect in the normal acid fermentation of milk, although they are constantly present in varying numbers, as can be demonstrated by appropriate means, and are of importance in certain phases of dairy manufacturing.

In any sample of milk the relative number of bacteria belonging to each of the first two groups is dependent upon the conditions surrounding production, especially with reference to cleanliness. The bacteria belonging to the first group come largely from the milk utensils and are also found in the dust of the barn and on the coat of the animal. The source of the second group is largely the fecal matter that gains entrance to the milk, although they are also found in the upper layers of the soil and on grain. They are introduced into the milk with the dirt. The cleaner the conditions of production, the smaller will be the number of these two groups of organisms found in fresh milk.

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* Prepared by E. G. Hastings.

The manufacture of the leading type of butter and of all kinds of cheese is dependent on the action of microörganisms, hence dairy manufacturing should be classed as a true fermentation industry. In all such industries one of the factors determining the quality of the product is the type of microörganism employed to produce the desired fermentation, and the importance of insuring the presence of desirable organisms, and the exclusion of harmful kinds is well recognized.

The most important properties of organisms employed in the fermentation industries are the physiological rather than the cultural or morphological, since the quality of the product is dependent on the by-products of the fermentation. Hence in characterizing the groups of acid-forming bacteria, the biochemistry of each group will be emphasized rather than the cultural and morphological characteristics of the members of the group.

*Characteristics of the Bact. Lactis Acidi Group.**—The organisms of this group are widely distributed in nature, as is shown by the constancy with which milk undergoes the characteristic fermentation produced by the members of the group.

The cells are oval in form, about 0.6μ to 1μ in length, and 0.5μ in diameter. The shorter cells appear nearly spherical, which, together with the fact that chains of cells often occur, has led some to classify them among the cocci and Kruse has applied the name *Strept. lacticus* to a member of the group. In milk the cells are usually in twos, the outer ends of the two cells being pointed. None of the group is motile; spores are not formed and capsules are often noted. The members of the group are Gram-positive.

The optimum temperature for growth lies between 30° and 35°, the minimum growth temperature ranging from 10° to 12°, while the maximum is 42°. They are to be classed as facultative aerobes. The growth on all culture media is marked by its meagerness; in the absence of a fermentable carbohydrate, no growth usually occurs; peptone favors the growth even in milk. In the case of freshly isolated cultures, the growth is almost invisible, on slopes of sugar agar appearing as small discrete colonies. On sugar agar plates the colonies are small, often surrounded by a hazy zone, and always occur below the surface of the medium. In lactose-agar stab cultures growth occurs along the entire line of inoculation, but there is no surface growth. No liquefaction of gelatin occurs. In bouillon the medium is uniformly turbid or it remains clear with a slight sediment. On potato, growth is slight or is absent. Milk is usually curdled within twenty-four hours at the optimum temperature by members of the group, although some fail to curdle the milk, since the maximum amount of acid produced is not sufficient to cause this phenomenon. Still others cause curdling in the presence of small amounts of acids, in which case a rennet-like enzyme may be present. No gas is produced in the fermentation of lactose, hence the curd formed in milk is perfectly homogeneous; it shows but little tendency to shrink and to express whey. In litmus milk the color is discharged from the entire mass of medium before curdling occurs, due to the reduction of the litmus to the colorless leuco-compound. Through the action of the oxygen of the air the litmus is slowly reoxidized and the pink layer, which immediately after curdling is but a few millimeters in depth, is slowly extended until the entire mass of curd has a uniform pink color. Saccharose, dextrose, maltose, and mannit are fermented.

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* Prepared by E. G. Hastings.

The maximum amount of acid produced by organisms that are most typical of the group is determined by the composition of the medium. It is often said that the organisms causing the normal souring of milk represent a group that can grow in a strongly acid medium. This is true as far as acid salts are concerned, but free acid totally inhibits growth. In a culture medium, which contains no substance that can combine with the acid formed and thus remove it from the sphere of action, no growth, or but very slight growth occurs. In sugar bouillon and in milk, the amount of acid formed is determined by the amount of substances in these liquids that can combine with the acid. In milk such compounds are the casein and some of the ash constituents, especially the phosphates. In normal milk, the maximum acidity attained ranges from 0.9 to 1.25 per cent calculated as lactic acid. If the content of neutralizing compounds per unit volume is varied by concentration, dilution, or by the addition of such substances as calcium phosphate, the maximum amount of acid produced by typical cultures will be changed. In sugar bouillon the maximum acidity produced rarely exceeds 0.25 per cent.

The fermentation of lactose is usually expressed as follows:

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3.$$

Thus 342 parts of lactose should yield 360 parts of lactic acid. The theoretical yield of lactic acid is never obtained, for the action of the organism on the carbohydrate is much more complex than is represented by the equation given. In the following table are given data obtained by a number of investigators.

These data signify that other compounds than lactic acid are formed in the fermentation of lactose by these acid-forming bacteria. Acetic acid ($CH_3.COOH$); formic acid ($H.COOH$); propionic acid ($C_2H_5.COOH$); traces of alcohols, aldehydes and esters have been found. The lactic acid formed is the dextro modification. It is believed that the fermentation is due to an enzyme, lactacidase, one of the intracellular enzymes that can be demonstrated only with difficulty.

Sugar content of milk, per cent.	Sugar fermented, per cent.	Lactic acid calculated, per cent.	Lactic acid found, per cent. of theoretical
4.54	0.60	0.632	89.56
4.96	0.56	0.590	98.13
4.94	0.65	0.684	97.89

Milk fermented by members of this group has a mild acid taste, an agreeable odor, and the curd can be so finely divided by agitation as to produce almost as perfect an emulsion as in raw milk. The organisms are to be classed as desirable from the standpoint of the dairy manufacturer, and the fermentation produced by them may be called a true *lactic* fermentation.

*Characteristics of the B. Coli-aerogenes Group.**—This group includes a considerable variety of organisms, which differ in morphology, in cultural characteristics and undoubtedly in the character and amounts of their by-products. They are more distinctly bacilli than the members of the preceding group; are motile or non-motile; none produces spores and they are usually negative to Gram's stain. The optimum growth temperature, 35° to 40°, is somewhat higher than for the preceding group, the vegetation range being 15° to 45°. They are to be classed as facultative anaerobes.

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* Prepared by E. G. Hastings.

The conditions for development are less narrow than for the *Bact. lactis acidi* group, growth occurring on all the ordinary culture media and in the absence of carbohydrates. Indol and hydrogen sulphide are often formed and nitrates are reduced. The growth is usually profuse, the colonies large and surface growth occurring in stab cultures. Gelatin is not usually liquefied.

Lactose, dextrose and saccharose are fermented, with the production of varying amounts of gas in which have been found carbon dioxide, hydrogen and methane. The maximum amount of acid produced in any culture medium is quite similar to that formed by the members of the previous group. The relative proportions between the non-volatile and volatile acids are far different, lactic acid comprising less than 30 per cent of the total acid formed, while volatile acids, such as acetic and formic, make up the remainder. Traces of succinic acid ($C_2H_4(COOH)_2$) and alcohol have also been found. The lactic acid is of the lævo-form.

Milk is usually curdled, although some members of the group do not produce enough acid to cause curdling. The amount of gas produced varies widely. In the case of those forms that cause curdling, the presence of gas is made evident by rents in the curd. If considerable gas is produced, the curd will be very spongy. When the acid formed is not sufficient to curdle the milk, the gas produced is likely to pass off unnoticed. The curd shrinks to a greater or less extent and thus becomes so firm that it is impossible to emulsify it again. The odor of the fermented milk is often offensive and the taste disagreeable and sharp. The organisms of this group are to be classed as undesirable and the fermentation produced by them cannot correctly be called a lactic fermentation.

Representatives of these two great groups of acid-forming bacteria are to be found in every sample of market milk in varying proportions. Both find in milk favorable conditions for growth, and the normal souring is produced conjointly by them, each producing its own specific products, the relative amounts of which are largely dependent on the number of each group that is originally introduced into the milk and on the temperature at which it is kept. The higher temperatures tend to favor the growth of members of the *B. coli-*

aerogenes group over that of the *Bact. lactis acidi* group The value of milk for butter and cheese is determined by the relative amounts of the products of the desirable and the undesirable acid-forming bacteria.

The difference in taste and odor between milk fermented by pure cultures of *Bact. lactis acidi*, and that which has soured spontaneously, emphasizes the difference in the products of the fermentations produced by the two groups of acid-forming bacteria.

*Characteristics of the Bact. Bulgaricum Group.**—The organisms of this group are to be classed as true lactic bacteria, since they produce almost exclusively lactic acid from the sugar fermented and only small quantities of other acids as formic, acetic, and propionic. They vary widely in form and size; but are usually large rods, 2μ to 3μ long and 0.5μ to 1μ wide. There is a tendency to form long threads. They are Gram-positive and when stained with methylene blue often show distinct granules in the cells; with Neisser's stain the appearance of some cultures is similar to that of the diphtheria bacterium. They are non-motile and do not form spores; capsules are seldom noted. The optimum growth temperature is from 40° to 50° and the minimum is asserted to be 25°, although for many members of the group it must be much lower.

The growth on all ordinary culture media is meager or is absent; the colonies are often microscopic in size and show radiating threads. Free acids do not inhibit development and the term *acidophilous* has been applied to the group. They grow slowly in milk, even at the optimum temperature, and curdling may not occur for several days; the curd is homogeneous and in litmus milk reduction occurs. The maximum amount of acid varies from 1.25 to 4.0 per cent. Some members of the group produce dextro-, others lævo-acid, and racemic acid is formed in some cases. The curd may be easily broken by agitation, and through the solvent action of the acid is partially dissolved. The organisms do not liquefy gelatin, but the casein of milk is partially changed into soluble decomposition products, as was first shown by de Freudenreich, and later confirmed by Hastings.

It has been supposed by many that this group was confined to and characteristic of certain of the fermented milks, especially those of eastern Europe and western Asia, such as Yogurt and Matzoon. Recent work has shown that this group is widely distributed in nature.

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* Prepared by E. G. Hastings.

Representatives of this group are found constantly in milk and other dairy products. Their presence in milk can be demonstrated by placing a sample of milk in a corked bottle, and incubating at 37°. The acidity of the milk increases rapidly at first, due to the growth of the members of the two previous groups. These ordinary acid-forming organisms are soon inhibited by the appearance of free acid, but the acidity of the milk nevertheless continues to increase slowly, and with this continued increase a change in flora is noted, the short, plump bacilli ceasing to predominate and long slender rods constantly increasing in numbers. The source of this group is undoubtedly the alimentary tract of the animal.

*Characteristics of the Coccus Group.**—This group is well represented by the bacteria which form the characteristic flora of the udder. They vary greatly in size and in other properties. They retain Gram's stain; many are chromogenic, the color ranging from a white to a deep orange. They grow slowly on all ordinary culture media, but the growth is not necessarily meager. Generally they are aerobic, although many grow under anaerobic conditions. Gelatin may be liquefied or not. Milk may or may not be curdled, the curd often resembling that formed by rennet-like enzymes. They produce no lactic acid, but only acetic, propionic, butyric and caproic acids, and hence cannot be classed as lactic bacteria.

BACTERIA HAVING NO APPRECIABLE EFFECT ON MILK.—This group is made up of many different forms. They produce no changes, during the normal life of market milk, which can be detected either by the eye or the taste. They do not develop very rapidly in milk, and some species gradually disappear while others increase in numbers. Many of the organisms in this group are chromogenic, orange and lemon yellows being among the more common forms. They are mostly cocci and do not liquefy gelatin. From the standpoint of the commercial milkman these organisms are of little significance and this is probably also true from the standpoint of the consumer.

THE CASEIN-DIGESTING OR PEPTONIZING BACTERIA.—These organisms digest the casein either with or without coagulation. Many of them cause the milk to curdle. The reaction is alkaline. The curdling agent is a rennet-like enzyme. They liquefy gelatin. Most of the organisms of this group are rods of various shapes and sizes, some of them being the largest rods found in milk. Some are motile and some non-motile. Some representatives of this group produce little or no odor, but many of the species develop very strong putrefactive odors. Barny or cowy odors or other off-flavors sometimes found in milk and dairy products may be caused by the action of this type of bacteria. They are associated with filth and their presence in milk indicates insanitary conditions of production or handling.

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* Prepared by E G. Hastings.

Pathogenic Organisms.—This group includes all those species which may gain access to milk, which are capable of causing specific diseases in human beings. They are of the greatest importance to the consumer. They do not appreciably affect the physical or chemical properties of the milk, or produce any changes in its appearance, flavor, or keeping quality which would indicate their presence. Some of them do not even develop in milk, as is the case with the *Bact. tuberculosis*. Others, as the diphtheria bacteria and typhoid fever bacilli, may grow in milk with great rapidity. This group also contains certain species which produce diarrhœal disorders, especially in infants and young children. Some of them are probably organisms which are also included in the peptonizing group. The specific pathogenic organisms, possibly with the exception of *Bact. tuberculosis*, get into milk, either directly or indirectly, from human patients suffering with the particular disease.

Factors Influencing the Development of Microörganisms in Milk

The number of microörganisms found in fresh milk shows its bacterial condition at that time, but it gives little idea of the organisms which may be found in the same milk at later periods. There are many factors to be considered if we wish to study the development of the various types which get into ordinary milk. These factors may be considered briefly under the following heads:

Initial Contamination.—Fresh milk varies widely in the number of organisms which it contains as a result of the conditions under which it has been produced. There are differences not only in the numbers of organisms but also in the species which may be found in different samples of fresh milk. Both of these factors are important in the later changes which may take place. The effect of numerical initial contamination may be seen in the following tables where

EFFECT OF INITIAL CONTAMINATION ON DEVELOPMENT OF BACTERIA AND KEEPING QUALITY OF MILK

Milk Having Moderately High Initial Contamination

Bacteria per c.c. in fresh milk	Bacteria 12 hours	Bacteria 36 hours	Hours to curdling
187,000	432,000	633,500,000	45

Milk Having Moderate Initial Contamination

Bacteria per c.c. in fresh milk	Bacteria 12 hours	Bacteria 36 hours	Hours to curdling
3,000	14,000	149,650,000	99

Milk Having Small Initial Contamination

Bacteria per c.c. in fresh milk	Bacteria 12 hours	Bacteria 36 hours	Hours to curdling
325	1,712	10,125,000	121

milk starting out with different numbers of organisms was kept under similar conditions until coagulation. Plate cultures made from these three samples show the relative development of the number of organisms.

These samples were all kept at a constant temperature of 21° and the difference in the numbers of bacteria and the curdling time can therefore be fairly attributed to the difference in the initial contamination of the three samples. All three of the samples showed a normal development of the lactic organisms, which constituted over 99 per cent of the total organisms present at the time of curdling. While this may be considered as showing the normal effect of the original contamination upon the milk, it is well to bear in mind the fact that there are many apparent exceptions due to some particular type of organism predominating and interfering with the normal development of the lactic types.

STRAINING.—The straining of milk is one of the most common operations in connection with its handling and is considered by most dairymen as one of the most essential from the standpoint of the quality of the milk. If milk is strained through cheese cloth or wire gauze much of the insoluble dirt can be removed. This has led to the general belief that straining improves the sanitary and keeping qualities of the milk.

The effect of straining on removal of insoluble dirt is shown by the following results of tests:

DIRT REMOVED BY PASSING MILK THROUGH TWO THICKNESSES OF FINE CLOTH
(Weight of insoluble dirt given in milligrams per liter of milk)

Experiment	Before straining	After straining	Per cent removed
No. 1	8.95	4.70	47.5
No. 2	5.55	4.95	10.8
No. 3	5.15	2.95	42.7
No. 4	2.45	0.20	91.8
No. 5	5.05	3.10	38.6

It may be noticed that even after straining the milk contained appreciable quantities of insoluble dirt which had passed through the strainer cloth. The difference in per cent of dirt removed in different samples is due to the nature of the dirt itself. The coarser the dirt the greater the proportion that will be removed by straining.

It is not true, however, that the keeping quality is necessarily improved by the simple process of straining. It depends largely upon the condition of the milk and the nature of the strainer. Not infrequently passing milk through a strainer not only fails to improve its keeping quality but actually injures it. This has been shown by a number of investigators. The effect of straining upon the germ content may be seen in the following figures where the milk was passed through a strainer composed of three thicknesses of fine cheese cloth supported by wire gauze.

EFFECT OF STRAINING UPON BACTERIAL CONTENT OF MILK

Experiment	Before straining, bacteria per c.c.	After straining, bacteria per c.c.
No. 1	3,600	3,600
No. 2	7,400	6,900
No. 3	12,800	10,500
No. 4	8,800	11,375
No. 5	2,800	2,700

The effect of straining upon the keeping quality is shown in the following experiments where the milk was strained through the same form of strainer mentioned above and the samples kept at constant temperature of 21° until coagulation.

EFFECT OF STRAINING UPON KEEPING QUALITY OF MILK

	Not strained, hours to coagulation	Strained, hours to coagulation
Experiment No. 1	42	42
Experiment No. 2	57	55
Experiment No. 3	35	35
Experiment No 4	89	54
Experiment No. 5	50	50

It will be seen that in no case was the keeping quality of these samples increased by the straining process while in some cases it was materially injured.

Cotton filters are more efficient than cheese cloth and in some cases the keeping quality of the milk may be improved by this process.

AERATION.—This is the process of exposing the milk to the atmosphere by allowing it to run over the surface of the aerator in a very thin film. If milk has been produced under such conditions that it has absorbed foreign odors, this process may be of value in getting rid of the absorbed odors, but from the bacterial standpoint the process of aerating is not desirable, since it gives one more opportunity for the milk to become contaminated with organisms from the atmosphere and from the aerator itself. It is possible to aerate milk under such conditions that the germ content will not be increased, but if aeration takes place in the cow stable or other place where the atmosphere contains dust the number of organisms will be greater after aeration than before, the amount of increase being proportional to the sanitary conditions under which the aeration is done. It is even possible that the milk may absorb foreign odors during the process of aeration and be of poorer quality than it was before. It is thought by many that the process of aeration is necessary in order to get rid of the so-called animal odors commonly found in milk. These odors are, however, not normal to the milk but are absorbed from the foul air in the stables or other sources. This is shown by the fact that some of the very finest quality of certified milk is bottled while still containing the animal heat with the least possible exposure to the air, tightly sealed at once and plunged into ice water. Such milk contains no suggestion of animal odor. Aeration may be of value in removing undesirable odors from milk which is not produced under good sanitary conditions, if done in an atmosphere free from all dust and odors, but it is not necessary for milk of good quality. The common belief that aeration is valuable is probably due to the fact that most aerators are coolers as well, and the beneficial results are due to the cooling and not the aeration.

Centrifugal Separation.—It is a common practice in some milk plants to pass the milk through a centrifugal separator or clarifier to remove any dirt which it may contain. This operation is effective for the removal of much of the insoluble dirt which may be in the milk, but it is of doubtful value from the standpoint of the bacterial content and the keeping quality of the milk. In spite of the fact that the separator slime is very rich in bacteria, the milk and cream as they come from the machine will normally show larger bacterial counts in agar and gelatin plates than will the milk before treatment, due of course to the breaking up of the bacterial groups. In some cases, however, there is an apparent decrease. The usual effect upon the germ content of passing milk through a separator or clarifier may be seen in the following tables:

Influence of Passing Milk through a Centrifugal Separator upon the Germ Content of the Skim Milk And Cream

	Plate count in whole milk	Plate count in skim milk	Plate count in cream
Sample No. 1	39,000	69,000	75,000
Sample No. 2	44,000	76,000	790,000
Sample No. 3	56,000	75,000	820,000
Sample No. 4	200,000	336,000	330,000

Effect of a Centrifugal Clarifier upon the Germ Content of Milk

Sample number	Plate count before clarifying	Plate count after clarifying	Numerical increase	Percentage increase
1	6,000	9,000	3,000	50
2	15,000	22,000	7,000	46
3	60,000	156,000	96,000	160
4	133,000	197,000	64,000	48
5	370,000	643,000	273,000	73

Similar results have been reported by Bahlman,* by McInerney,† and by Sherman.‡ Some investigators, especially Hammer‖ and Marshall and Hood,§ have reported results showing that in some lots of milk the plate count from the clarified milk is less than in the original milk. This is shown in the following data given by the last named authors.

BACTERIA IN COMMERCIAL MILK BEFORE AND AFTER CLARIFICATION

Sample No.	Number of bacteria in 1 cubic centimeter of unclarified milk	Number of bacteria in 1 cubic centimeter of clarified milk	Per cent increase
1	250,000	900,000	260
2	100,000	200,000	100
3	75,000	65,000	−13
4	20,000	50,000	150
5	5,000	12,000	14
6	125,000	70,000	−44
7	130,000	400,000	207
8	25,000	48,000	92
9	20,000	35,000	75
10	350,000	250,000	−28
11	30,000	40,000	33
12	40,000	50,000	25
13	30,000	20,000	−33
14	10,000	10,000	
15	16,000	33,000	106

In the case of the increased counts they do not mean that there is an actual increase in individual bacteria in these samples due to the action of the separator or clarifier. What it does mean is that the small clusters or groups of organisms, as they exist in the whole milk are thrown apart by the centrifugal force and therefore develop a larger number of individual colonies in the plate cultures in spite of the fact that large numbers of organisms are thrown out in the slime.

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* Bahlman, Clarence. Milk Clarifiers, Am. Jour. of Public Health, 1916, Vol. VI, No. 8, 1916.

† McInerney, T. J. Clarification of Milk. Cornell Agr. Exp. Sta. Bull. 389, April, 1917.

‡ Sherman, James M. Bacteriological Tests of Milk Clarifier. Jour. of Diary Science, 1917, Vol. I, No. 3, p. 272.

‖ Hammer, B. W. Studies on the Clarification of Milk. Iowa Agr. Exp. Sta. Bull. 28 1916.

§ Marshall, C. E. and Hood, E. G. Clarification of Milk. Mass. Agr. Exp. Sta. Bull. 187, Nov., 1918.

TEMPERATURE.—The temperature at which milk is kept is one of the most important factors determining the development of its microbial content. Every one at all familiar with milk knows that it spoils very quickly if allowed to stand at warm temperatures. If, however, the milk is held at temperatures of 10° or lower, its keeping quality is greatly increased. Most of the ordinary species of organisms which gain entrance to milk do not grow rapidly at temperatures of 10° or lower. There are, however, certain species which will grow with considerable rapidity at temperatures below 10°, especially some of the spore-bearing non-acid forms. If the temperature of the milk is allowed to rise above 10°, the growth of the common species increases rapidly The influence of temperature upon the development of bacteria may be seen in the following experiment where a given lot of milk was thoroughly mixed and divided into seven portions, which were then held at the temperatures indicated for twelve hours, at the end of which time they were plated for the total germ content.

EFFECT OF DIFFERENT TEMPERATURES UPON THE DEVELOPMENT OF BACTERIA IN MILK

Temperature maintained for 12 hours		Plate count per c.c. at end of 12 hours	Hours to curdling at 21°
C.	F.		
4.5°	40°	4,000	75
7°	45°	9,000	75
10°	50°	18,000	72
12.5°	55°	38,000	49
15.5°	60°	453,000	43
21°	70°	8,800,000	32
26.5°	80°	55,300,000	28

The fresh milk showed a count of 5,000 per c.c. and curdled in fifty-two hours at a temperature of 21°. The curdling time of these samples was determined by placing them at a constant temperature of 21° at the close of the twelve-hour period and holding them at this temperature until coagulation took place. The difference in time of curdling therefore is due to the maintenance of the special temperature for twelve hours only and not for the entire period up to the time of curdling.

PASTEURIZATION.—The term pasteurization is used to designate the process of heating milk to a temperature sufficient to destroy a portion of the bacteria, including the pathogens, and then cooling it to a temperature which will prevent the rapid development of the organisms that are left. The temperatures commonly used for this purpose vary from 60° to 85°. The length of time the milk is exposed to the high temperature may also vary from a few seconds to thirty minutes, depending upon the method employed. The two chief purposes for the pasteurization of milk are to destroy any pathogenic organisms which the milk may contain and to increase its keeping quality. The purpose for which the pasteurization is done will determine the method used. In commercial pasteurization, where the chief purpose is to destroy the lactic organisms and thus improve the keeping quality of the milk, the method used is that known as the "flash" or instantaneous method, where the milk is subjected to a high temperature for a few seconds only and then cooled. In this method of pasteurization varying degrees of efficiency are obtained, depending upon a number of factors, chiefly the bacterial condition of the milk to be pasteurized, the degree of heat and the length of the exposure and the temperature to which the milk is cooled. By this method, it is possible to destroy a large percentage of the organisms in the raw milk, and materially increase its keeping quality, but the temperature and time to which any particle of milk is exposed cannot be accurately controlled, and this method cannot be depended upon to kill all of the disease-producing organisms which may be in the milk. This method has been largely abandoned for the pasteurization of market milk.

Under present conditions of the market milk business where the chief purpose of pasteurization is to render the milk free from disease-producing organisms, the so-called "holding" method is employed. This consists in raising the temperature of the milk to about 60° to 63° and holding it at this temperature for a period of twenty to thirty minutes. If this method is properly done, most of the organisms except certain spore forms should be killed and the milk at the end of the pasteurizing process contain only a small percentage of its original germ content.

Formerly it was believed that heating milk to a high temperature killed all the lactic acid organisms, and favored the subsequent growth of other more undesirable species, but more recent studies on the bacterial flora of milk, pasteurized by the "holding" method, have shown that some strains of the lactic acid bacteria can survive the relatively lower temperatures used in this method, and that the later development of the different groups of bacteria is similar to that in raw milk of equal bacterial grade.

Pasteurization at the temperatures used in the holding process does not seem to cause any injurious chemical changes in the milk constituents, or affect its digestibility.

Proper pasteurization gives a valuable means of rendering the milk supply for our cities reasonably free from pathogenic microörganisms, but, in order to insure this safety, the work must be carefully done, and all later contamination avoided. Preferably, the work should be done under expert, municipal supervision. Undoubtedly the ideal method is pasteurization in the sealed bottle which is to be delivered to the consumer, since this method reduces to the minimum the danger of subsequent contamination.

Pasteurization must not be regarded as a substitute for care and cleanliness or a means of renovating old or dirty milk otherwise unfit for use, but rather as an additional means of protecting the consumer against disease-producing microörganisms in the milk supply.

The Use of Chemicals.—The addition of certain chemicals to milk will retard the growth of bacteria. The chemicals most commonly used for this purpose are calcium hypochlorite, borax and formalin. While the keeping quality of milk may be materially increased by the use of such chemicals, their use has been opposed by health authorities and is contrary to the Pure Food Laws. If milk is handled with any degree of care, there should be no need for the use of chemical preservatives. They are simply a means of counteracting the unsanitary conditions of the production and handling. The same results can be obtained by cleanliness in the production of the milk and the use of low temperatures for preventing the contamination and subsequent growth of the bacteria in the milk. The developments in the production of clean milk of the past few years have illustrated very clearly that the use of chemical preservatives is not necessary.

Normal Development of Microörganisms in Milk

The flora of any particular sample of fresh milk is determined by the conditions under which it is produced. In stables where extreme cleanliness is practised the flora may be practically limited to those species which occur in the udder of the cows, but under ordinary conditions there will be in addition to the normal udder types such others as may occur on the cow's body and in the dust and atmosphere of the stables. Market milk, therefore, when first obtained from the cow ordinarily contains a mixed flora, the different types present depending upon the sanitary conditions under which the milk is produced.

The future development of this initial flora is largely dependent upon the temperature at which the milk is kept. If the milk is held at temperatures between 10° and 21° there will result what may be considered as the normal development of milk fermentations. These changes may be divided for convenience into four periods or stages.

First Stage. Germicidal Period.—It has been shown by a number of investigators that instead of an increase in the numbers of bacteria in fresh milk there is normally a decrease in the number during the first few hours after its production. The rapidity of this decrease and the length of time over which it extends seem to be determined largely by the temperature at which the milk is kept. The higher the temperature the more rapidly the number of organisms decreases and the more quickly the end of the germicidal period is reached. If the temperatures are kept fairly low the rate of decrease is much slower but the decline will extend over a considerably longer period. This is shown by the following examples given by Hunziker.

Table Showing the Germicidal Action in Cow's Milk

Name of cow	Milk, warm and fresh	Temp.* of milk	After 3 hours	After 6 hours	After 9 hours	After 12 hours	After 15 hours	After 24 hours	After 32 hours	After 48 hours
May.......	1,212	40°	1,080	1,220	1,040	1,020	1,120	1,360	1,040	400
		55°	1,260	1,400	1,500	1,462	1,360	1,080	3,500	17,740
		70°	1,000	1,340	1,860	3,460	3,460	64,000	800,000	
Ida........	5,120	40°	4,400	4,260	3,620	3,700	3,900	4,000	3,900	3,840
		55°	3,900	3,460	2,980	2,800	2,920	3,260	3,220	3,240
		70°	3,560	2,120	1,880	1,880	1,240	4,960	58,400	
Julia.......	1,345	40°	1,170	1,070	1,120	870	1,120	990	1,060	1,080
		55°	1,080	990	980	1,400	1,080	1,080	3,110	68,800
		70°	1,000	1,000	1,200	5,600	17,720	1,600,000		

The exact reason for this decline is at present not well understood. Some investigators believe that milk possesses a certain germicidal action or property which results in the destruction of a portion of the organisms found in the milk at the outset.

* Fahrenheit.

The work of other investigators seems to show that the so-called germicidal action is felt by certain species and not by others as is indicated by the following sample.

Age of milk	Total bacteria	Acid bacteria	Per cent. acid bacteria	Liquefying bacteria
Fresh	12,550	1,250	10	200
3 hours	12,250	2,000	16	200
6 hours	19,650	2,250	23	800
9 hours	56,900	20,250	36	550
12 hours	114,250	68,400	60	1,900

This would seem to indicate that the decrease in number is due not so much to a definite germicidal property possessed by the milk as to the gradual dying out of certain species which for some reason do not find the milk a suitable environment for development, while other types, finding the milk suitable to their needs, develop uniformly from the start.

Rosenau and McCoy found that the germicidal properties of milk were destroyed by boiling or by heating it above 80° and that lower temperatures destroyed it for certain organisms. These workers also found that there was marked agglutination of the organisms in raw milk and conclude that this accounts for the decreased number of colonies developing in plate cultures and that the germicidal action is therefore more apparent than real.

SECOND STAGE. PERIOD FROM END OF GERMICIDAL ACTION TO TIME OF CURDLING.—The period following immediately after the germicidal action is characterized by the rapid development of the lactic organisms. Under normal conditions this group develops much more rapidly than any other type. Not only do they increase rapidly in actual numbers but their percentage also rises rapidly. There may be a continual increase in numbers in the other species, but their growth is much less rapid than that of the *Bact. lactis acidi* type. As this period advances certain of the miscellaneous types may cease to grow entirely. During this time the gas-producing acid organisms of the *B. coli* and *Bact. lactis aerogenes* type may develop more or less rapidly, but if the milk is held at temperatures not much above 20°, the *Bact. lactis acidi* type will develop much more rapidly, so that by the time the milk becomes sour and curdles, this type will constitute 99 per cent approximately of the total number in the milk. From the standpoint of the milk consumer milk ceases to be of value when the end of this period is reached, but there are further developments which are of importance in certain lines of dairy manufactures, notably cheese making.

THIRD STAGE. PERIOD FROM TIME OF CURDLING UNTIL ACIDITY IS NEUTRALIZED.—At the time milk curdles it contains enormous numbers of the lactic bacteria. The number usually runs into the millions and may be even higher than one thousand million per c.c. By the time the coagulation takes place the acidity of the milk is so high that the growth of the lactic organisms is checked and from this time on their number decreases with more or less rapidity.

During the period following the curdling certain other types of organisms which have remained more or less dormant in the milk during the earlier stages now begin to grow. The organisms especially important in this stage are *Oidium lactis*, certain species of molds, and yeasts. These organisms are able to grow in a highly acid medium, and as a result of their development the acid is decreased until the milk finally presents a neutral or alkaline condition resulting from the decomposition of the proteins in the milk.

FOURTH STAGE. FINAL DECOMPOSITION CHANGES.—The reduction of the acidity affords favorable conditions for the growth of certain types of organisms which have remained in the milk during the earlier stages but have been practically dormant. In this fourth stage the conditions are suitable for the growth of the liquefying and peptonizing bacteria and they now grow rapidly, causing the decomposition of the casein. The changes resulting from this type of organisms are of special significance in cheese making and are discussed more fully in another chapter.

ABNORMAL FERMENTATIONS IN MILK

GASSY FERMENTATION.—It frequently happens that instead of the normally rapid development of the *Bact. lactis acidi* type of organisms in the milk, other acid producers develop rapidly, with the production of more or less gas. The organisms most prominent in this type of fermentation are the *B. coli communis* and the *Bact. lactis aerogenes* types. This group of organisms contains a number of varieties, some of which produce little or no gas while others develop large amounts. Their action in milk is usually accompanied by disagreeable odors and flavors. They grow readily in the presence of air and therefore develop abundant colonies on the surface of plate cultures. This distinguishes the members of this group quite clearly from those of the true lactic group which grow chiefly below the surface of the medium. The members of this group do not form spores, but certain varieties are quite resistant to heat and will oft times survive pasteurizing temperatures which completely destroy the *Bact. lactis acidi* group. They grow most rapidly at high temperatures, between 20° and 37°.

Sweet Curdling Fermentation.—This phenomenon is caused by a variety of organisms which cause the milk to coagulate without the production of acid. The coagulation is brought about by a rennet-like enzyme produced by this type of bacteria. The resulting milk is either neutral or alkaline in reaction. Usually the coagulation of the milk is followed by the digestion of the casein as a result of another enzyme which is also produced by these bacteria. The coagulation caused by these organisms is slower than in the case of the acid formers and the curd is usually soft and mushy as compared with the curd formed in the normal acid fermentation. The members of this group get into the milk from and along with dust and dirt associated with unsanitary conditions. Some of the species produce spores and are not killed by the ordinary methods of pasteurization. This fact accounts for the occurrence of sweet curdling of pasteurized milk. This group of organisms is unable to develop rapidly in the presence of the lactic bacteria and for this reason we do not commonly get the sweet curdling of raw milk. The presence of these organisms is evidence of insanitary conditions. Frequently they develop very disagreeable flavors in the milk.

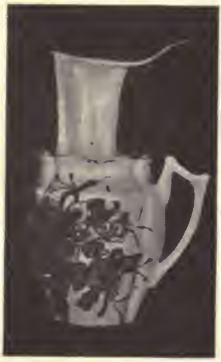


Fig. 143.—Ropy cream lifted with a fork. (*After Ward.*)

Ropy or Slimy Fermentation.—One of the most common milk infections causing trouble to the milk dealer is that which causes a ropy or slimy fermentation of milk. This is sometimes spoken of as stringy milk (Fig. 143). Several species of organisms are capable of producing this condition. These organisms grow most freely in the presence of an abundant supply of oxygen and for this reason the cream usually becomes slimy before any changes are apparent in the underlying layers of milk. *B. lactis viscosus* is perhaps the most common species in this group. The slimy condition in the milk is supposed to be the result of a very viscid capsule surrounding these organisms (Fig. 144). Representatives of this group are quite resistant to heat and frequently pass uninjured through the methods of cleansing and scalding used under ordinary dairy conditions. Because of this, dairy utensils once infected may become a constant source of infection.

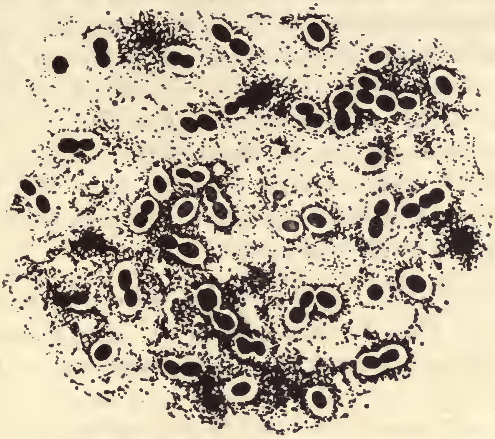


FIG. 144.—*Bacillus lactis viscosus* from a milk culture. (*After Ward.*)

This trouble can be effectively stopped by a thorough scalding of all utensils coming in contact with the milk.

BITTER FERMENTATION.—Bitter flavors in milk may be the result of bacterial changes after the milk has been drawn, or due to certain feeds which the cows have consumed. If the cows are allowed to eat certain kinds of vegetation, such as "rag weed" and certain other plants, they may impart a bitter taste to the milk, in which case the abnormal flavor will be apparent when the milk is fresh and usually becomes less pronounced as the milk becomes older, because of the volatile nature of the substances causing the bitterness. Most of the cases of bitter milk and cream, however, are due to the growth of certain types of bacteria in which case the bitterness increases in intensity with the age of the milk. Some of the species capable of producing bitter milk grow at quite low temperatures, which accounts for the fact that the most trouble with bitter flavors is found in milk and cream which has been held at low temperatures for some time.

Alcoholic Fermentation.—The bacteria as a group are not able to act on the milk sugar and produce alcohol, but it sometimes happens that yeasts get into the milk in sufficient numbers to ferment the milk sugar, producing appreciable amounts of alcohol. To the milk handler this trouble is not usually serious but the action of the yeasts is frequently of considerable importance in the cheese industry.

Other Fermentations.—It frequently happens that a considerable variety of disagreeable flavors and odors develop in milk. These may be due to the direct absorption of odors from the foul stable atmosphere or strong-smelling feeds, such as silage; or they may be, and no doubt frequently are, the result of the growth of certain types of bacteria which have entered the milk from dirty surroundings. The growth of some of these organisms is frequently the cause of the so-called cowy and stable odors and flavors.

Commercial Significance of Microörganisms in Milk

Relation of Dirt Contamination to Germ Content.—To the commercial milkman bacteria are of importance only as they influence the length of time the milk will keep in a salable condition. The consumers do not want milk that is sour or has unpleasant flavors and odors. In order to sell his milk, therefore, the milkman must avoid the presence of these undesirable conditions, and in proportion as he recognizes the relation between germ life and the quality of his product, will he pay attention to the presence and development of microörganisms in his milk. In like manner, the presence or absence of dirt contamination is important from the commercial standpoint since it bears a relation to the bacterial count, and, therefore, affects the keeping properties of the milk. Under normal conditions there is a fairly direct relation between the amount of visible or soluble dirt and the number of bacteria found in any given lot of fresh milk. This relation may be shown by the following samples taken from four different milk producers:

Producer	Number of samples	Average mg. dry dirt per liter	Average number bacteria per c.c.	Average hours to time of curdling
A........	5	51.5	115,000	175
B........	16	58.8	273,600	78
C........	21	70.0	428,600	75
D........	17	71.9	949,400	68

This relation does not always hold for the reason that a gram of one kind of dirt may contain infinitely more organisms than an equal amount of some other kind. The difference in the solubility of various forms of dirt always causes apparent discrepancies in this normal relation. In the majority of cases, however, the relation shown in the above examples will hold reasonably true in the case of fresh milk. There is also a general relation between the number of bacteria in fresh milk and the length of time it will keep before souring and curdling. In this case the relation is in inverse ratio, the smaller the initial contamination, the longer the keeping time, and *vice versa*. This relation is also shown in the table given above. There are many irregularities, however, in this relation because of differences in the flora of fresh milk. It may frequently happen that a sample of milk containing a relatively high number of organisms will not sour as quickly as another sample with a smaller original germ content. The associative action of the different species of organisms is an important factor here. In making comparisons of this sort, it is, of course, necessary that the different samples be held at the same temperatures.

MILK AS A CARRIER OF DISEASE-PRODUCING ORGANISMS

It is not the purpose of this chapter to discuss in detail the diseases which may be carried by milk, but a chapter on bacteriology of milk would be incomplete without a brief discussion of this important subject.

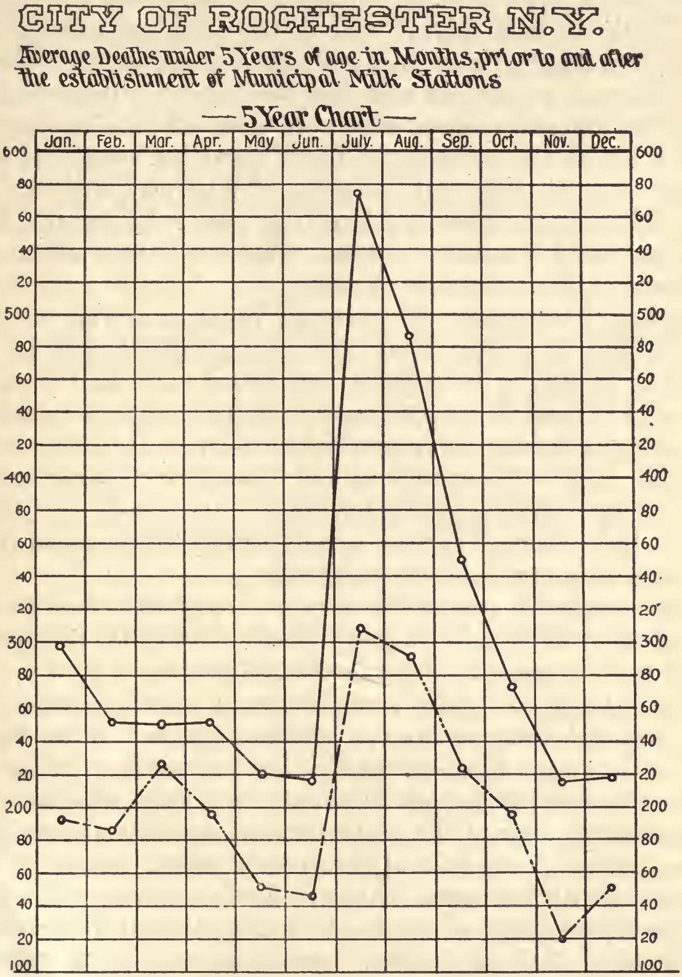
From the standpoint of their relation to the health of the consumer the microörganisms in milk may be divided into three groups on the basis of whether they are beneficial, inert or injurious to health.

Acid Forms.—The preservative properties of sour milk have been known since very ancient times. Its use as a preservative for meat, eggs and other perishable food products demonstrates the value of sour milk as a means of preventing decomposition. It has also been known for a long time that sour milk has a certain therapeutic value because of the action of the lactic bacteria in preventing harmful fermentations in the digestive tract. More recently the work of Metchnikoff has shown the usefulness of sour milk both for the treatment and prevention of intestinal disorders by inhibiting the development of the putrefactive bacteria in the digestive tract. In view of the value of sour milk for preventing certain forms of disease and its inhibiting action on certain undesirable organisms the *Bact. lactis acidi* type of bacteria must be regarded as beneficial organisms, and from the standpoint of the health of the consumer their presence in the milk is to be welcomed rather than discouraged. As the value of sour milk drinks becomes better known the importance of this group of milk bacteria will be more fully recognized.

Neutral or Inert Forms.—In ordinary milk there is a large class of bacteria which, so far as known, have no appreciable effect either upon the composition of the milk or the health of the persons consuming it. This group includes a number of species, many of them being coccus forms, some of them appearing in plate cultures as chromogenic colonies. They grow more or less freely in milk, depending upon the conditions, but they are usually held in check by the acid-forming bacteria and do not constitute a very important part of the flora of normal milk. They are, therefore, of little significance from the practical standpoint except as they indicate the conditions under which the milk has been produced and handled.

Injurious Organisms.—The diseases which may be carried by milk are of two classes.

Epidemic Diseases.—The human diseases most commonly carried by milk are typhoid fever, diphtheria and scarlet fever and occasionally other diseases such as septic sore-throat, cholera and foot-and-mouth disease. The first three are by far the most important of this group. The outbreaks of typhoid fever which are traceable to milk occur most frequently. There is a large accumulation of data showing the occurrence of epidemics caused by infected milk. An epidemic caused by the milk supply has certain characteristics which distinguish it from epidemics resulting from other causes. A considerable number of cases of the particular disease will appear almost simultaneously and will be distributed along some particular milk route. Usually the epidemic stops as suddenly as it began except for a few secondary cases contracted from those first taken. The source of the disease organisms is a human patient suffering from the disease. The infection of the milk may be direct, as when a sick person handles a milk, or it may be indirect as when a person caring for a patient also works about the milk. In other cases it may be caused by contamination of the water used in washing the utensils or by cows wading in water of infected streams and getting the organisms on their body whence they fall into the milk pail at milking time. The return of milk bottles from the sick room sometimes is the means of infecting the milk supply.



CITY OF ROCHESTER N.Y.

Average Deaths under 5 Years of age in Months, prior to and after the establishment of Municipal Milk Stations

—5 Year Chart—

FIG. 145.

Unfortunately the specific organisms of these diseases grow readily in milk and a small infection is all that is necessary to render the milk dangerous by the time it reaches the consumer.

Non-epidemic Diseases.—There is another class of diseases which may be carried by milk which are not characterized by a sudden out break, and for this reason are not so readily recognized as being associated with the milk supply. One of these diseases, namely tuberculosis, is caused by the specific, well-known organism, *Bact. tuberculosis,* which may get into the milk from the udder of a tuberculous cow or by the organisms which have been given off from the digestive tract of the animal becoming scattered about the stable and finally getting into the milk with particles of dust and filth. In some cases the milk may become infected by persons having the disease being permitted to handle the milk. Fortunately for mankind *Bact. tuberculosis* does not multiply in milk.

Regarding the danger of contracting tuberculosis from the use of milk there is at present some difference of opinion, but the consensus of opinion at the present time seems to be that there may not be very great danger for healthy adults, but that a considerable percentage of the cases of tuberculosis of children may be traced from the milk supply. Fortunately none of these specific disease organisms produce spores and the temperatures used in the process of pasteurization by the "holding" method are sufficient to destroy any of the disease bacteria known to be carried by milk.

There is another class of disorders not so well defined as the above but which are nevertheless of great importance from the standpoint of public health, especially of young children and also to some extent of adults. This group includes such disorders as infantile diarrhœa, summer complaint, cholera infantum and other disorders of the digestive tract. The organisms producing these troubles doubtless belong to the group of putrefactive bacteria which come from filth. Some of the gas producers and some of the peptonizers are probably responsible for these troubles. Shiga isolated from a large number of cases of infant diarrhœa a bacterium which he named *Bact. dysenteriæ,* but in general the specific organisms responsible for these intestinal troubles are not well known. Their importance, however, is shown by the relation of the germ content of milk to infant mortality (see Fig. 145).

Bacteriological Analysis of Milk

The development of our knowledge of the relation of bacteria to the wholesomeness of foods has led to a study of the bacterial content of milk as a means of determining its purity. The methods used for this purpose have followed quite closely those of the water bacteriologists.

For many years, dairy bacteriologists have endeavored to determine the number of organisms in milk by plating it into nutrient agar or gelatin. By this method the number of colonies developing in the plates is assumed to represent the germ content of the milk. But even when the best methods are employed, the plate count represents only the approximate and not the exact number of bacteria in any lot of milk. It should also be borne in mind that such counts are always underestimates, because of the fact that not all species will develop in any given medium or incubation temperature. The careful worker can recognize certain types of bacteria in plain media, but the addition of blue litmus solution to either agar or gelatin, greatly assists in the differentiation of types and species.

The Direct Microscopic Method.—The plating method is expensive because of the large amount of time and materials needed. It is not possible for one person to handle a large number of samples at one time. In routine work in the city laboratories this labor has been a serious drawback to this method. In order to decrease the labor and give greater possibilities to the work Stewart devised a method by which the bacterial condition of milk can be studied by direct microscopic examination. His purpose was to determine only the species present, but later Slack and still more recently Breed developed the method for determining the approximate numbers as well as the general species present in a given sample of milk.

Leucocytes.—The microscopic examination of milk sediment revealed the fact that frequently a sample would be found which showed the presence of leucocytes in greater or less numbers. The presence of these cells was regarded as important because it was assumed that they showed the presence of inflammation and pus formation in the udders of the cows producing the milk.

Several methods have been used for determining the leucocyte content of milk. "The Smear Sediment" and "Blood Counter" are methods which more strictly belong to laboratory practices and will not be considered in this place.

Bacteriological Milk Standards

The relation of the bacterial content of milk to its wholesomeness has led to the adoption of certain standards by the boards of health in our cities. These standards recognize the fact that the germ content of milk in the large cities is greater than in the smaller ones because of the greater distance from which it is shipped and its age on arrival to the city. New York City in 1900 adopted a maximum limit of 1,000,000 per c.c. Later Boston established a limit of 500,000, Chicago 1,000,000 from May to September, inclusive, and 500,000 from October to April, inclusive and Rochester 100,000. Other cities have made similar standards.

Stokes' standard for the number of leucocytes permissible in normal milk was 5 per field of the $1/12$ objective in his smeared sediment preparation. Bergey found so many samples running above this number that he made the limit 10 cells per field and felt that no milk containing more than this number should be used for food. Later Slack raised the limit to 50 cells per field. The reason for changing the standard was due partly to the larger numbers found as a result of improved methods but more especially to the discovery that milk from apparently healthy cows normally contains leucocytes in excess of the first standards set.

With the development of the dairy score card, there was a decided tendency to place emphasis on the sanitary conditions at the farm rather than on the germ content of the milk. But it was soon discovered that the farm score did not necessarily show the true condition of the milk, and at present, the tendency seems to be toward placing more confidence in the germ content as the best measure of the true conditions of production and handling. However, the fact must be recognized that our methods of bacteriological analysis are not sufficiently accurate to justify the bacteriologist in passing judgment concerning the quality of any milk supply on a single analysis. In order to secure results which are at all trustworthy, a *series* of analyses must be considered.

It is held by some that a numerical standard is of little value since the actual number of organisms present in a given lot of milk may not be a correct measure of its wholesomeness. For this reason some cities pay little attention to the numbers of bacteria present but base their standards wholly on the species and the quality of the milk is judged on the presence and numbers of streptococci, *B. coli*, leucocytes, sediment. Milk is passed or condemned on the basis of any one or combination of these conditions.

In recent years there has been a tendency to combine these two standards using the total germ content as a measure of the care the milk has had and the presence or absence of certain groups or species as an indication of the occurrence of pathological conditions in the cows producing the milk. The practice in most city laboratories now is to make use of both the numbers and the species present in determining the quality of the milk supply.

VALUE OF BACTERIOLOGICAL MILK STANDARDS AND ANALYSES

Regarding the value of bacteriological standards for milk there is still some difference of opinion among milk bacteriologists. The germ content of any lot of milk is largely dependent upon three factors: the number of organisms getting into the fresh milk; the temperature at which it is kept; the age of the milk when analysis is made.

The high bacterial count in any lot of milk may be the result of any one of these conditions or a combination of them. A high count means that there has been carelessness either in the production, resulting in high initial contamination, or in the subsequent handling permitting a rapid multiplication of the organisms, or that the milk is old.

On the other hand, milk with a low germ content can be obtained only where the original contamination is small and the milk has been held at low temperatures. A low count, therefore, means care both in the production and later handling of the milk.

While the germ content may be regarded as a general index to the care the milk has received, it may not at all indicate its wholesomeness. A high count may be the result of the rapid growth of the lactic bacteria, in which case the milk may be perfectly safe and wholesome. On the other hand, the count may be quite small but contain pathogenic species. The bacteria count is valuable as showing the sanitary conditions of production and handling, but much care should be used in the interpretation of such results. In some ways a direct microscopic examination of the milk sediment is much more satisfactory. The skilled analyst can recognize certain types which may indicate the sanitary quality of the milk. With sufficient experience one can recognize streptococci, certain other groups and leucocytes. The presence and abundance of one or more of these groups may indicate the nature of the original contamination and the existence of diseases in the udders of cows. If rightly interpreted the information thus obtained is of much value. The weakness of this method lies in the fact that it is not possible to recognize all types of disease organisms. In a smear preparation it is not possible to differentiate between pathogenic and nonpathogenic streptococci or between *B. coli* and certain other types. The presence of unusual numbers of streptococci and pus cells may indicate the existence of disease in the cows and when this condition is found in the milk it is often possible to trace it back to the farm, locate the diseased cow and prevent her milk from being used for human consumption.

The tendency at present is to combine the quantitative and qualitative analyses and the results thus obtained in the hands of the careful worker are of much practical value in controlling the quality of a city's milk supply.

CHAPTER II*

THE RELATION OF MICROORGANISMS TO BUTTER

Butter is the fat of milk that has been largely freed from the other constituents of milk by the processes of creaming and churning. If milk is allowed to stand, the fat, which is in the form of minute globules, accumulates in the upper layers of the milk because its specific gravity is much lower than that of milk serum. In modern practice the fat is concentrated in a portion of the milk by passing the milk through a cream separator. In the rapidly revolving bowl of the separator the centrifugal force exerted is many times greater than that of gravity and the fat is rapidly and efficiently removed. The cream, which is obtained by these methods, contains varying amounts of fat which is further concentrated, by subjecting it to agitation in the churning process. The globules of fat cohere to form larger and larger masses until the entire amount of fat is brought into a single mass, the butter.

Types of Butter

Sweet-cream Butter.—If little or no increase in the acidity of the milk or cream develops, previous to churning, the butter will have certain marked characteristics and is called *sweet cream* butter. It is especially characterized by its low flavor, since it has only the flavor of the fat of milk which is not marked. This is usually known as the *primary* flavor of butter. Sweet-cream butter is also marked by the rapidity with which it undergoes decomposition changes, especially when it is made from raw cream.

Sour-cream Butter.—If the cream is allowed to undergo the acid fermentation, the butter will differ markedly both in degree and kind of flavor from that prepared from sweet cream, and as a rule its keeping qualities are much better than those of sweet-cream butter. This type of butter is made throughout northern Europe, England and her colonies, and in America. It may be said to be the standard butter of the world since it is the type made in all the great dairy countries. Sweet-cream butter is made especially in southern Europe, and in limited amounts in other countries.

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* Prepared by E. G. Hastings.

The intensity and kind of flavor of butter is thus dependent on the acid fermentation of the milk or cream. It is not believed that the fat undergoes any changes during the acid fermentation of the milk which could produce the flavor of sour-cream butter, but rather that the increase in flavor is due to the absorption by the butter fat of certain of the compounds formed in the acid fermentation. It is not essential that the fat be present during the acid fermentation in order to impart flavor to the butter. If sweet cream is mixed with sour milk and churned at once, the flavoring compounds are absorbed by the fat from the fermented milk, and the butter will have much the same flavor, both as to intensity and kind, as though the fat had been present during the fermentation. The churning of a mixture of sweet cream and sour milk is used commercially and is identical with the methods employed by the manufacturers of oleomargarine and renovated butter to impart flavor to the flavorless fats they employ. It is impossible to recognize these substitutes for butter by their flavor since it is identical with and derived from the same source as the flavor of butter.

In the past many ideas have been expressed as to the source of the flavor of butter; some have asserted that it is due, in part, to the decomposition of the proteins of milk by proteolytic bacteria. Both practical experience and experimental work have demonstrated the connection between the acid fermentation of milk and the flavor of butter, and it is certain that what is now considered the finest type of butter can be made from cream in which only acid-forming bacteria (see Chap. I) have grown.

FLAVOR OF BUTTER

CONTROL OF BUTTER FLAVOR.—The commercial value of any sample of butter is largely determined by its flavor. If it is lacking in flavor and aroma, or if it has a poor flavor, it brings a low price. The importance of being able to control the flavor, both as to degree and kind, in the manufacture of butter has increased greatly in recent years, because of the introduction of the creamery system, which has largely supplanted the making of butter on the farm. The financial success of any creamery is largely dependent upon the ability of the butter maker to control the flavor of the product, so that it shall be uniform from day to day. It is asserted that one of the factors in the remarkable invasion of Denmark into the butter markets of the world is the uniformity of the Danish butter, not only from a single creamery, but from all the creameries of the country. To the Danes we owe the most improved methods for the control of the flavor of butter.

The other points, texture, color and salt, which the judge of butter takes into consideration, can be easily controlled, since they are due to mechanical operations. The flavor, on the other hand, is due to the by-products which are formed by microörganisms in the fermentation of the milk and cream, and which are absorbed and held by the fat. If any of the products formed possesses a disagreeable taste or an offensive odor, the flavor and aroma of the butter will be impaired. It is thus evident that the control of the flavor of butter is dependent on the control of the acid-forming bacteria that ferment the milk and cream. This is the problem of the modern butter-maker and the modern methods seek to give him this control, to enable him to eliminate the undesirable bacteria, *B. coli* and *Bact. aerogenes*, the second group,* and to insure the predominance of the desirable bacteria, *Bact. lactis acidi.* This general statement is not to be interpreted as meaning that all bacteria that injure the flavor of butter are to be included in the group mentioned, for many other types of bacteria, when present in milk in large numbers, may injure the flavor of the butter prepared from it.

The acid fermentation of the cream is most frequently called the ripening of cream and sour-cream butter is frequently called ripened-cream butter. The ripening of the cream not only increases the flavor of the product, but it enhances its keeping quality. The ripening of the cream also aids in the mechanical process of churning, the sour cream churning more easily and with less loss of fat in the butter milk.

KINDS AND NUMBERS OF BACTERIA IN CREAM.—The number and kinds of bacteria found in cream are dependent upon the number and kind in the milk from which the cream is obtained. The cream will, however, contain a greater number of bacteria per unit volume than the milk, since the immense number of fat globules passing through the milk serum carry mechanically a considerable proportion of the bacteria of the milk into the cream. This phenomenon is to be noted in gravity creaming, but to a much greater extent in the removal of the cream by use of the separator.

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* See Chap. I. Div. IV, in which the groups of bacteria are considered.

Spontaneous Ripening of Cream.—By this expression is meant the fermentation of the cream by those acid-forming bacteria that have, from one source and another, gained entrance to it, but which have not been intentionally added. Under these conditions the butter-maker can exert but little control over the fermentation. A very considerable part of the butter made from such cream has an excellent flavor, because at the temperature at which cream is usually kept, *Bact. lactis acidi* and related organisms are the primary factors concerned in its fermentation and their by-products produce desirable flavors in butter. It has often been asserted that the highest type of butter can be made only from spontaneously ripened cream.

As the cream from many farms was assembled at a creamery for the manufacture of butter, it became evident that some means of controlling the type of fermentation in the cream was needed. If the milk had been produced under clean conditions, and had been received at the creamery before the acid fermentation had gone on to any extent, and if the cream was then kept at temperatures most favorable for the lactic bacteria, the product was likely to be of good quality, but such ideal conditions did not always obtain. Cream containing a large proportion of harmful bacteria, or in an advanced state of fermentation, or possessing an undesirable flavor was often received, and the butter-maker could not control the quality of the product under such conditions.

Use of Cultures in Butter Making.—As the science of microbiology progressed and the rôle of microörganisms in all kinds of fermentation became known, it became evident that the control of the causal organism is an important factor in determining the quality of any product of the fermentation industries. In the manufacture of butter, the first step in this direction was the addition of some fermented milk, cream, or of buttermilk to the cream to be ripened. In this manner the number of acid-forming organisms in the cream was greatly increased, and the fermentation went on more rapidly and in a more definite direction than without such additions, as the bacteria added were largely of the desirable group, *Bact. lactis acidi*. The addition of fermented milk to accelerate the souring of cream antedates by many hundred years the science of bacteriology.

The next logical step in the development of the process was the use of the same types of bacteria from day to day. Cultures of these were obtained by allowing a quantity of milk to sour, and if it had the desired flavor, a small amount of it was added to another quantity of milk that had been heated, in order to destroy the acid-forming bacteria it contained. By the daily preparation of some heated milk, and the inoculation of it with the soured milk previously prepared, the butter-maker could use the same types of bacteria for an indefinite time for addition to the cream.

It had been found by Hansen that, in order to control the flavor of beer, pure cultures of yeasts must be used for the fermentation of the wort. The success of this method in the brewing industry led to the introduction of pure lactic cultures for the fermentation of cream. The use of such cultures was suggested independently by Storch, a Danish bacteriologist and by Weigmann, the director of the dairy experiment station at Kiel in Germany, in 1890. Many cultures were isolated and tested as to their effect on the flavor of butter. Those found to be desirable could be maintained in the laboratory, and could be furnished to butter-makers to be used and propagated in a manner similar to the method employed with the impure and less constant home made starters. The pure cultures of lactic bacteria are widely used at present in the butter-producing countries of the world and their use is being constantly extended, as butter makers come to recognize the importance of controlling the ripening of cream.

It was found that the butter made from cream ripened by pure lactic cultures did not possess as high a flavor as did the finest butter made from naturally ripened cream. This led to the search for organisms that could be used alone, or together, with the lactic bacteria, and which should give the high flavor desired. Such cultures were found, but their use did not prove practical, either because they did not maintain their properties on continued cultivation, or because of their effect on the keeping quality of the product. The difference in flavor in the case of butter made from naturally ripened cream and that from cream ripened by pure lactic cultures is undoubtedly due to the products of the *B. coli-aerogenes* group.

The acid in spontaneously soured milk is very evident to the taste when the acidity is 0.6 per cent and above; the volatile acids formed by the members of the colon-aerogenes and coccus groups impart a sharp, pungent taste. In milk of like acidity fermented by pure cultures of *Bact. lactis acidi*, the acid is scarcely evident to the taste and there is no sharpness, due to the absence of volatile acids. This same difference appears in the butter made from the two kinds of milk.

The low flavor of the butter made from cream ripened by pure cultures was one of the factors that prevented the rapid introduction of the cultures in this country. The demands of the butter market have changed and the mild flavored butter, which is now considered to be the finest, can be made by the use of pure cultures in the fermentation of pure sweet cream.

COMMERCIAL CULTURES.—In this country the preparation and distribution of cultures for the ripening of cream is largely in the hands of commercial firms; hence, the term "commercial culture" is applied to them. The different pure cultures are propagated in the laboratory of the maker; they are sent out either as liquid cultures, a small mass of milk or bouillon inoculated with the organism, or in a dry form, the latter being prepared by mixing a culture of the organism with an inert substance, such as milk sugar, milk powder, or starch, and drying at a low temperature. In a liquid the organisms are exposed to the effects of their own by-products, and the vitality of the culture is rapidly lost. Such cultures must be used when fresh in order to give good results, and they cannot be kept in stock by the manufacturer or dealer. The resistance of *Bact. lactis acidi* to desiccation is great; it thus lends itself to the preparation of the dry cultures, in which the organisms remain in a dormant condition and retain their vitality for long periods.

Most of the cultures now sold are pure, as this term is used in bacteriology, still others contain non-acid-forming organisns intentionally added or introduced accidentally during the process of preparation. If the lactic bacteria are present in such cultures in large numbers, the impurities are usually of small practical significance. In the past so-called "duplex" cultures have been sold which were supposed to contain an acid-forming organism and a second organism that was to enhance the flavor of the product. Such cultures are no longer sold.

For the propagation in the creamery the contents of the container purchased are added to a small mass of milk that has been heated to destroy all non-spore-forming bacteria and other microörganisms; the milk, after being inoculated, is incubated at favorable temperatures and when curdled can be used for the inoculation of the second and larger quantity. The process of inoculating a quantity of milk is carried out daily. It is impossible for the butter-maker to propagate the culture in such a way as to maintain its original purity, but if the milk is heated sufficiently, if all utensils are sterilized, and if the culture is kept at a temperature that is especially favorable for the organism, the contamination that may occur will not injure the culture for practical work. The cultures propagated under such conditions gradually deteriorate and recourse must be had sooner or later to a fresh culture. The contamination that is of the greatest significance is undoubtedly that with other acid-forming bacteria rather than with the forms that remain in the milk after heating.

Many of the cultures gradually lose their fermentative properties, and do not form acid rapidly and in sufficient amounts to insure exhaustive churning and to produce the desired degree of flavor in the product. Cultures frequently become slimy or ropy on propagation. This is not necessarily due to contamination with specific slime-forming organisms but rather to a change in the lactic organism itself. Such an abnormality usually persists for only a short period and the conditions that govern its appearance and disappearance are not known. It is asserted by practical butter makers that the development of too high an acidity in the cultures as they are propagated in the creameries permanently impairs the value of the culture.

The cultures are propagated in skim-milk. Where this is not available, unsweetened, condensed milk or milk powder have been employed. Efforts have been made to grow the bacteria in some other kind of medium than milk, but without success. The starter is said to be ripe or in the best condition for use soon after curdling, or when the acidity is 0.5 to 0.7 per cent, as at this time it contains the maximum number of living cells. The practical man thus uses the curdling as an indication of the ripeness of the starter. The curdled milk should show no free whey, and the curd should be easily broken up to form a creamy mass that can be uniformly incorporated with the cream. The temperature of incubation and the amount of initial inoculation determine the rapidity with which the acid fermentation will progress, the maker seeking to regulate these so that the culture shall be ripe at the desired time each day.

USE OF PURE CULTURES IN RAW CREAM.—The cream as it reaches the creamery contains a greater or less number of acid-forming bacteria that ultimately will cause it to ripen and the flavor of the butter will be due to the by-products of the mixture of bacteria. If, through the addition of a pure culture, the relative number of organisms that are known to be favorable is greatly increased, the flavor of the product should be improved. This has been found to be true in practice and it is now believed that pure cultures are of value not only in the ripening of sweet cream, but that the addition of a relatively large amount of starter to cream that is already fermented will enhance the value of the butter.

USE OF PURE CULTURES IN PASTEURIZED CREAM.—It is evident that the maker has but imperfect control over the fermentative processes when raw cream is treated with a pure culture. To insure more perfect control the destruction of the contained bacteria and the subsequent inoculation of the cream with a pure culture is indicated. The introduction of the process of pasteurization of cream for butter making was due to Storch. In Denmark this method is used almost exclusively. It has been introduced into the other dairy countries of the world and is constantly spreading. Pasteurization combined with the use of the pure culture represents the highest type of modern butter making, and where the raw product can be obtained in a fresh condition the butter-maker has perfect control over the bacteria that cause the ripening; hence he can control the flavor of the butter, both qualitatively and quantitatively.

The intensity of flavor of butter is dependent upon the amount of acid that is developed in the cream or more correctly on the ratio between the amount of fat and the by-products of the acid fermentation. If these by-products are small in amount, as in cream having a low acidity, the flavor of the butter will be low. If the acidity is allowed to reach the maximum, the flavor will be much higher. Thus the maker can control the intensity of flavor of butter as accurately as he can the kind of flavor. With rich cream, the acidity that can be developed is small and the ratio between the fat and the products of fermentation is low; thus, the flavor of butter made from very heavy cream is certain to be low.

Pure Cultures in Oleomargarine and Renovated Butter.—It was previously mentioned that the manufacturer of butter substitutes employs the same methods to impart butter flavor to his products as does the butter-maker. The oleomargarine manufacturers employ pure cultures of lactic bacteria for the fermenting of milk that is mixed with the fats they employ. The same practice is followed by the manufacturer of renovated butter. Many of the creameries of the western states receive cream that is shipped long distances, and is collected from the farms but once or twice a week. It is thus in an advanced state of fermentation when it reaches the creamery. In order to prepare from this grade of cream, which often has a most undesirable flavor, a merchantable product, various means are employed to remove the flavoring substances and to replace them with desirable flavors from the pure cultures. The acidity may be reduced by the addition of lime so that the cream can be pasteurized; the cream may be aerated by passing air through it, or it may be mixed with water and reseparated. After such treatment it is mixed with a large proportion of milk fermented by a pure culture and churned. The resulting product is constantly sold as the highest grade of creamery butter.

Abnormal Flavors of Butter.—Most of the abnormal flavors of butter are traceable to the partial replacement of the desirable acid-forming bacteria with other types of microörganisms Many samples of butter having abnormal flavors have been examined, and the organisms believed to be the cause isolated and studied but it cannot be said that any particular group of microörganisms can be associated with any of the abnormal flavors met. It is asserted that "oily" butter, *i.e.*, that having the taste of machine oil, is caused by bacteria and by microörganisms that decompose the fat, as *Oidium lactis*, yeasts, and liquefying bacteria. Organisms of the *B. coli* group that produce a turnip-like flavor in butter have been described by Weigmann. The flavors of putrid butter, fishy butter and also many other abnormal flavors have been ascribed to bacteria.

Other abnormal flavors may be due to the presence in the milk of certain aromatic principles contained in the feed and excreted in the milk. Cabbage, turnips, and other plants impart their characteristic taste to the milk and butter.

DECOMPOSITION PROCESSES IN BUTTER

Butter is a finished product at the time it is removed from the modern churn and all subsequent changes are likely to cause more or less deterioration. The specific causes of these changes are not well known but it is very evident from a study of the conditions that favor or retard the appearance of the flavors, characterizing these changes, that biological factors are concerned. When raw cream is used, sweet-cream butter has very poor keeping qualities. As the proportion of acid-forming bacteria in butter is increased, either by the fermentation of the cream, by the addition of pure cultures, and through the use of the latter in connection with pasteurization, the keeping qualities are enhanced. Of the butter made from ripened cream, that prepared from cream, handled in a clean manner, and thoroughly pasteurized and ripened with a pure culture of *Bact. lactis acidi*, has the best keeping quality. If fresh, sweet, clean cream is pasteurized, the butter will have better keeping quality than when made from the same cream pasteurized and ripened with a pure culture. This is evidence that not only the bacteria other than *Bact. lactis acidi* are harmful, but that this organism, that has usually been considered without influence on the keeping quality, must be classed as one of the factors in the decomposition of butter.

It has been shown that the bacterial content of the water used for the washing of the butter has an influence on the keeping quality. If the water is of surface origin and contains the bacteria peculiar to these types of waters, its influence may be marked and some method of treatment must be followed. Filtering or heating the water has been resorted to, the latter with marked success. A pure water will contain so few bacteria that they will not exert any noticeable influence on the keeping quality of the butter.

Storage temperature also has a marked influence on the deterioration changes in butter. Modern butter-storage rooms are kept below 0°F.; the butter deteriorates slowly during storage at these temperatures, but on removal undergoes change much more rapidly than would have been true before storage. Another factor that is of influence in the keeping of butter is the amount of salt used. In salted butter, the contained water is a concentrated brine; in such a medium most forms of bacteria are unable to grow. Small packages deteriorate more rapidly than large ones, because the proportion of the mass of butter exposed to the air is relatively greater. Exposure to light is also claimed to exert a harmful influence. Antiseptic substances such as borax and boric acid have a marked effect on the deterioration changes. The New Zealand and Australian butter exported to the English markets is treated with preservatives.

A large amount of experimental work has been done in order to determine the effect of specific organisms on the keeping quality of butter. The results obtained have not been definite and it is not certain that the organisms employed are constantly concerned in the deterioration changes. It is very probable that both bacteria and molds exert an influence. The chemical changes that take place in the spoiling of butter are no better known than are the causal factors. It has been asserted that there is a decomposition of the glycerides with a resulting increase in free acids. It has been shown that this does not always occur; that a butter may be in an advanced state of decomposition and its content in volatile acids not be higher than when fresh. Two types of changes are usually distinguished, rancidity and the appearance of a tallow-like odor. The latter may be due to purely chemical factors, while the former is quite certainly biological.

Moldy butter is a frequent trouble encountered by the butter-maker. If the butter is not salted, molds may develop just below the surface. The most usual form of mold to appear is one with black hyphæ; the slightest development of which will be evident on the butter. In the case of salted butter, mold on the butter itself is very rare, due apparently to the concentration of the brine in the butter. The parchment paper in which print butter is wrapped and with which the butter containers are lined is an excellent substratum for mold growth. If the papers and containers are badly contaminated with mold spores, or if they have been kept under such conditions as to permit of a limited amount of growth before they are used, the development of the mold on the paper after it is brought into contact with the butter is likely to be rapid, even at low temperatures, and the butter is likely to reach the market in an objectionable condition. The paper may be rendered free from molds by placing it in water which has been heated to at least 80°. Butter tubs are scalded, steamed, or soaked in brine or treated with a dilute solution of formalin in order to destroy the mold spores that may be present. The most efficient manner of preventing trouble is to coat the inside of the butter tub with paraffin. This prevents trouble from the container but not from the paper. Mold spores or vegetative hyphæ are likely to be present in the cream. Those which find themselves on the immediate surface of the butter may grow. This source of trouble can be overcome by pasteurization of the cream.

PATHOGENIC BACTERIA IN BUTTER

If the milk contains pathogenic bacteria, they are certain to pass into the cream and be incorporated in the butter. It is not believed that butter is an important agent in the distribution of the organisms of tuberculosis and typhoid fever, although both are able to exist in salted butter for over two months. Foot-and-mouth disease is said be caused in humans by the use of butter made from the milk of infected animals, but this may still be regarded as a mooted question.

CHAPTER III*

THE RELATION OF MICROORGANISMS TO CHEESE

General

Cheese consists of the fat and casein of milk, together with the insoluble salts; however, along with these constituents are carried some of the moisture of milk, in which are dissolved small quantities of sugar, albumin, and salts. The amount of moisture and soluble constituents found in cheese is determined by the amount of whey incorporated in the curd.

In the process of making cheese, it is necessary to curdle the milk, thus enabling the separation of the casein and fat from the milk serum. Two methods are employed to accomplish this purpose, and, as a result, two types of cheeses are produced.

Types of Cheese

These types may be designated as "*Acid-curd Cheeses*" and "*Rennet-curd Cheeses.*"

Acid-curd Cheeses.—The curdling may be accomplished by allowing the milk to undergo acid fermentation, either spontaneously through the action of the normal flora of the milk, or through the addition of pure lactic cultures. Most acid-curd cheeses are ready for use as soon as the whey has been removed by draining and the curds salted. Acid-curd cheeses are not commercially important. They are made for local consumption and are to be classed as a form of sour milk. They owe their flavor to the products of the acid fermentation, especially lactic acid. The moisture content is high, which, together with the acid reaction, favors the growth of molds and yeasts. These biological agents may soon spoil the cheese.

Rennet-curd Cheeses.—All of the important varieties of cheeses are made by the use of rennet for the curdling of the milk. Over four hundred kinds of rennet-curd cheeses are made, but only twelve to fifteen are of great commercial importance. With few exceptions, they are made from cow's milk. From the same raw material—milk, rennet, and salt—therefore, a wide variety of products, differing in texture, taste and odor, is obtained. This fact indicates the importance of biological factors in the changes the curd undergoes during the ripening process.

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* **Prepared by E. G. Hastings.**

The rennet-curd cheeses may be divided into: (1) *hard cheeses;* (2) *soft cheeses;* the initial difference is largely in the amount of whey left in the curd during the making of the cheese. The two great groups of rennet-curd cheeses gradually merge into each other in varieties that by some are classed as hard cheese, by others as soft cheese.

The rennet-curd cheeses, as a rule, are at first tough and rubber-like in texture. The curd, which is not easily digested, is quite insoluble in water and is devoid of flavor and aroma. The curd must pass through a complete series of chemical and physical changes, which alter its texture, solubility, and digestibility, and give to it a flavor and aroma by which the different kinds of rennet-curd cheeses are especially to be differentiated.

In the hard cheeses the factors concerned in these changes act in a uniform manner throughout the entire mass of the cheese, making it possible to manufacture such cheeses in any desired size. In the case of the soft cheeses, the ripening changes are largely due to agents which grow only on the surface; the products of such agents by means of diffusion gradually affect the entire mass. In order that this may take place within a reasonable time, it is essential that these cheeses be made in small sizes. Then, too, the soft texture of such cheeses makes it impossible to handle them commercially in large sizes.

Conditions Affecting the Making of Cheese

Quality of Milk.—In the curdling of milk by rennet the solid bodies present in the milk are retained in the curd, thus the fat globules are held, as are also the bacteria. The latter continue to grow as they would have done in the milk except that growth must take place in the form of colonies as in the solid culture media of the bacteriologist. The bacteria, however, produce the same fermentation in the curd as they would have done in the uncurdled milk.

The butter-maker can control, through pasteurization and the use of pure lactic cultures, the fermentation of the cream. The pasteurization may be so efficient as to destroy all non-spore-forming bacteria since the quality of the product will not be impaired by the use of temperatures approximating the boiling point. The cheese-maker is much more dependent on the original quality of the milk, since it has not been found possible to make most of the important varieties of cheeses from pasteurized milk. If undesirable forms of microörganisms are present in the milk, they will pass into the cheese and there produce their harmful effects. Through the addition of pure cultures of

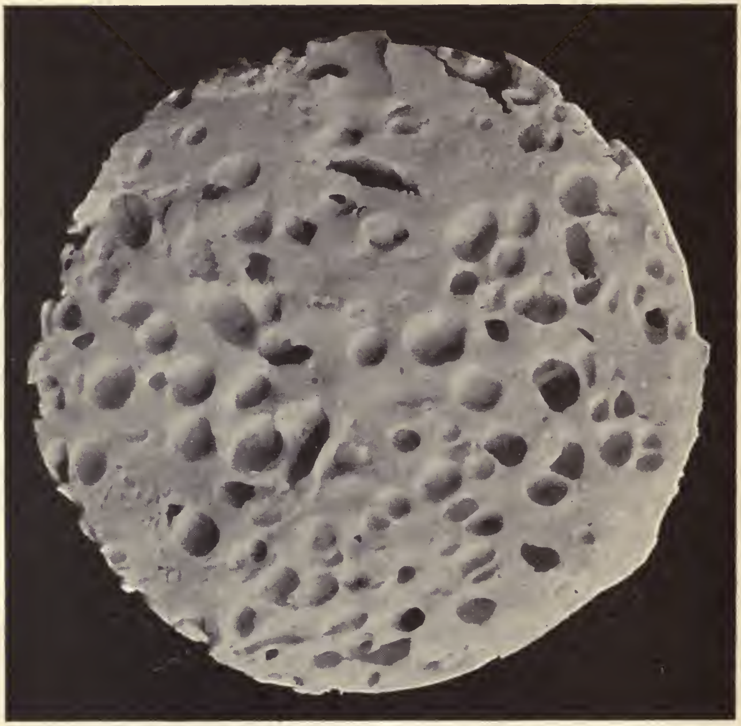

FIG. 146.—The type of curd obtained from milk in which the acid-forming flora consists largely of organisms of the *B. coli-aerogenes* group. Many gas holes and few irregular shaped, angular, mechanical holes due to imperfect "matting." (*Original.*)

Bact. lactis acidi to the milk, the proportion of desirable bacteria can be increased and a partial control of the fermentation thus secured.

TESTS FOR THE QUALITY OF MILK.—Methods by which the cheese maker can determine, in a rough manner, the kinds of bacteria present have been devised. The bacteria most dreaded and most frequently present are those of the *B. coli-aerogenes* group.

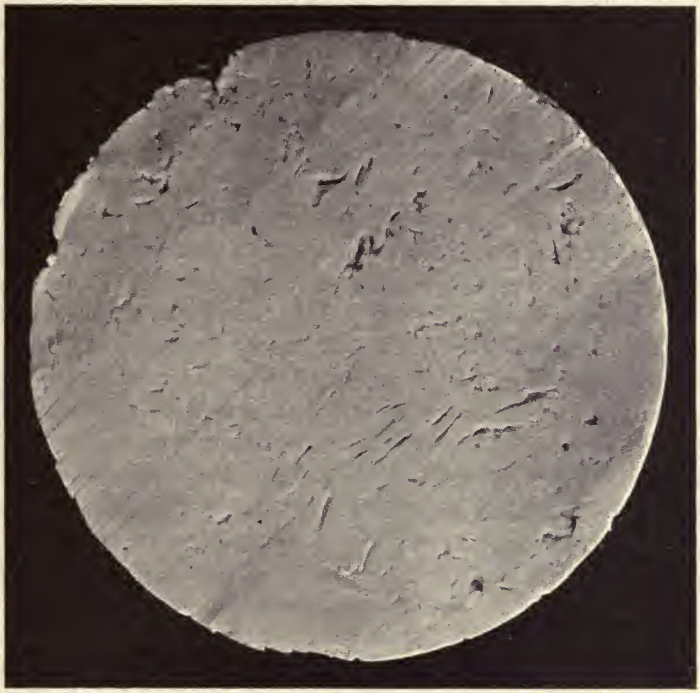

FIG. 147.—The type of curd obtained from milk in which the acid-forming flora consists almost wholly of *Bact. lactis acidi.* No gas holes and no marked mechanical holes as the curd has "matted" almost perfectly. (*Original.*)

The method most frequently used for their detection consists in incubating a sample of the milk to be tested at temperatures ranging from 35° to 40° for a few hours and noting the type of curd that is formed. Milk suitable for cheese making should show the solid curd characteristic of the *Bact. lactis acidi* group, while gassy curds or soft and partially digested curds are indicative of bacteria that are likely to be harmful in the cheese.

An improvement over the fermentation test of foreign origin has been devised by Babcock and Russell and is known as the *Wisconsin Curd Test.* It has for its basis the same principle as the simple fermentation test; however, a modification is introduced; the milk is curdled by the addition of rennet and the curd is cut and drained to free it from the whey as completely as possible.

The undesirable organisms most likely to be present in milk are those of the *B. coli-aerogenes* group; therefore the jars containing the curds should be kept at temperatures, 35° to 40°, that will favor their development. The great advantage of the *Wisconsin Curd Test* is its greater delicacy, since the bacteria are concentrated in a small volume, and thus their presence is more evident than would be the case in the larger mass of curd obtained when no rennet is added. The curd can also be removed from the jar, cut, tasted, and its texture determined, all of which aid in judging the quality of the milk. The curd should have a clean acid odor and taste; it should be free from sliminess on the surface, and possess a uniform texture. Such a curd can be obtained only in the presence of a considerable number of lactic bacteria. Very clean, fresh milk is likely to give an undesirable result, since milk always contains microörganisms which will grow rapidly at the high temperature in the absence of the acid-forming bacteria and which will usually produce undesirable flavors in the curd. This fact should be kept in mind in the testing of market milk.

Ripening of Milk.*—The methods for the determination of acidity in milk have very considerable limits of error. It is not possible to detect any increase in acidity until the number of acid-forming bacteria has increased to hundreds of thousands per c.c. Originally it was thought that no acid was produced by the growth of the acid-forming bacteria during the initial stages of their development. This period during which bacterial proliferation was taking place, but without an apparent increase in acidity, was known as the "period of incubation." It is now certain that this rests upon our inability to detect small increases in acidity. The Cheddar cheese-maker desires milk that shall contain such a number of acid-forming bacteria that during the operations that are carried on in the first part of the cheese-making process large amounts of acid shall be formed in the curd. He thus wishes to know, as accurately as can be determined under the conditions found in the factory, the number of bacteria in the milk which he is to use. This information is gained either by the titration of the milk with a standard alkali solution or by determining the time required for the curdling of a definite quantity of milk at a definite temperature by a known quantity of rennet. Very much smaller increases in acidity can be detected by the so-called rennet test than by titrating the milk. If the milk shows the desired acidity when it reaches the factory, the making process is immediately begun. If the milk is too sweet, or in other words, too low in its bacterial content, bacterial growth is favored by warming the milk to temperatures most favorable for the lactic bacteria, 30° to 32°, and by the addition of pure cultures of *Bact. lactis acidi* which are identical in nature and the method of propagation with those used in butter making. The development of a slight acidity is known as the "ripening" of milk.

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* In order to illustrate the rôle of microörganisms in the making and ripening of cheeses, a somewhat detailed summary of the present knowledge concerning their action in Cheddar cheese will be given. Many of the factors concerned in the ripening of this kind of cheese also function in the ripening of other rennet cheeses. In their description only such additional factors need be considered as are not active in Cheddar cheese.

In order to insure proper rennet action the maker of Cheddar cheese desires the milk to have an acidity of about 0.2 per cent. He thus wishes milk in which an appreciable amount of acid has been formed.

CURDLING OF MILK.—Under the influence of a favorable temperature and the slight acidity, the milk is quickly changed by the rennin* to a firm, jelly-like mass that is cut, with appropriate knives, into small cubes. The curd encloses over 80 per cent of the bacteria in the milk. The same factors that favor the curdling of the milk favor the shrinking of the curd and the expulsion of the whey from the cubes. The development of acid within the curd is rapid, due to the concentration of large numbers of bacteria in a small volume and to the favorable environment. During the six to eight hours that elapse between the curdling of the milk and the pressing of the curd, the increase of acidity is over 0.1 per cent per hour. The following table gives the acidity of milk and the whey expressed from the curd at various stages in the making of a typical Cheddar cheese.

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* The rennet used in cheese-making is obtained by extracting the abomasum, the true digestive stomach of the calf, with a solution of sodium chloride. The extract contains two enzymes, a clotting or curdling enzyme, *rennin*, and a proteolytic enzyme, *pepsin*,

Acidity of milk before adding rennet	0.20–0.21 per cent.
Acidity of whey immediately after cutting curd.	0.14–0.145 per cent.
Acidity of whey when removed from the curd	0.16–0.18 per cent.
Acidity of whey when curd is packed	0.24–0.30 per cent.
Acidity of whey when curd is milled	0.65–0.75 per cent.
Acidity of whey when curd is salted	0.90–1.10 per cent.

Manipulation of the Curd.[*]—The curd particles at first show little tendency to cohere; but, as the acidity increases, the nature of the curd changes, and, when the whey is removed, the pieces of curd soon cohere and ultimately form a single mass in which the original cubes of curd cannot be detected. The fusion of the curd particles is known as "matting" and is an important step in the Cheddar process. The lack of acid formation within the curd prevents matting while the curd is in the vat, and may even render difficult the fusion of the particles under pressure. The nature of the change which the curd undergoes at this stage in the manufacture is not well understood, but probably is due to a combination between the paracasein and the lactic acid, the resulting compounds differing from the paracasein in physical properties and in solubilities.

Ripening of Cheese.—Cheese in ripening undergoes profound physical and chemical changes under the influence of a number of factors, which for purposes of discussion may be divided into two groups: those by which the content of soluble nitrogen in the cheese is increased and the digestibility enhanced; and those which cause the formation of flavoring substances. During the ripening of the cheese the maker can do little toward the control of the factors which ultimately determine its commercial value. As in butter, the flavor is the most important characteristic of the ripened cheese and the most difficult to control.

Theories of Cheese Ripening.—Many theories have been advanced to explain the changes that occur during the ripening process. Duclaux, a French microbiologist, studied the bacterial flora of Cantal cheese by aid of the crude methods available before the introduction of the gelatin-plate method. By the use of the dilution method, using bouillon as the nutrient medium, he isolated a number of kinds of spore-forming bacteria. The organisms formed two enzymes, one a curdling enzyme related to rennin, the other a proteolytic enzyme to which was given the name *casease*. A chemical study of the by-products of the organisms, when growing in milk, revealed a number of compounds that had previously been found in ripe cheese, such as leucin, tyrosin, and the ammonia salts of acetic, valeric and carbonic acids. The cultures often possessed a cheese-like odor. These facts led Duclaux to believe this class of organisms was responsible for the ripening of the hard cheese in question. The generic name *Tyrothrix* was applied on account of the supposed relation to cheese. This term is still found in current bacteriological literature. The methods employed by Duclaux were such as favored the growth of the liquefying, rather than the acid-forming bacteria. To the latter more recent investigators have devoted attention.

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[*] Cheddar cheese.

The theory that the proteolytic bacteria function in the ripening of hard cheese has been more recently emphasized by Adametz. It is sufficient to say that the number of spore-forming proteolytic bacteria in cheese is not sufficiently large, nor is their presence so constant that any importance can be attached to them. Any agent to be considered as a factor in the ripening process must be present in every cheese in sufficient numbers to account for the change for which it is considered responsible. Such agents should be capable of demonstration. It should be remembered that, by following the rules laid down by the practical maker, a normal cheese can invariably be made, hence the factors of importance in the ripening must be constantly present in the milk or rennet. It is doubtful whether the liquefying bacteria will satisfy this requirement. It has been shown by de Freudenreich that such organisms, even when added to milk in large numbers, exert no influence on the ripening of hard cheese, since the conditions within the cheese are not such that growth can occur.

De Freudenreich, a Swiss microbiologist, by the aid of modern methods, demonstrated the constant presence of certain classes of acid-forming bacteria in Swiss cheese, and to them ascribed an important rôle in the ripening of this hard cheese. He was led to this conclusion by their great numbers in the fresh cheese, and by the fact that cheese made from milk drawn under aseptic conditions, which thus contains no lactic bacteria, do not ripen; through the discovery, also, that certain of the lactic bacteria, predominating in Swiss cheese, those of the *Bact. bulgaricum* group, exert a solvent effect on the casein of milk, although they are devoid of action on gelatin.

Babcock and Russell demonstrated the presence of an inherent proteolytic enzyme in milk, to which the term *galactase* was applied. This enzyme can be demonstrated by preserving a sample of fresh milk with chloroform or other mild antiseptic. At 37° curdling occurs in three to four weeks; the content of soluble nitrogen in the milk is slowly augmented. The presence of this proteolytic enzyme, together with the fact that a normal cheese cannot be made from milk in which this enzyme has been destroyed by heat, led these investigators to consider this inherent enzyme of milk an important factor in cheese ripening.

*Present Knowledge of Causal Factors.**—The rôle of certain factors in the ripening of Cheddar cheese has been established beyond doubt by the chemical and bacteriological investigations of recent years. It is certain that acid-forming bacteria are essential factors in the ripening of this kind of hard cheese, and probably of all kinds of rennet cheeses.

As has been shown the growth of acid-forming bacteria is rapid during the making of Cheddar cheese. The growth continues during the pressing and subsequent thereto; the maximum number of lactic bacteria is found when the cheese is one to five days old. As many as 1,500,000,000 per g. of the moist cheese have been demonstrated.

Causes of Proteolysis.—The proteolytic action of rennet extract on the paracasein of cheese was demonstrated by Babcock and Russell, and by Jensen. This property is due to the fact that rennet extract also contains the enzyme *pepsin*, which for its action outside the body requires conditions similar to those which obtain in the stomach; in other words, the presence of sufficient acid to activate it. The hydrochloric acid secreted by the walls of the stomach acts as the activating agent in the body. The acidity resulting from the fermentation of the sugar in the curd is sufficient to activate the pepsin. Under its influence the paracasein is partially converted into soluble decomposition products such as albumoses and peptones. In the absence of acid-forming bacteria no acid is formed; consequently the pepsin does not become active and no proteolytic effect is produced. Under these conditions the curd remains tough and elastic and the solubility is not increased. It is thus evident that acid-forming bacteria are essential factors in cheese ripening. The pepsin of the rennet extract and the galactase suffice to account for the initial proteolysis of the paracasein. Since neither of these enzymes forms ammonia, which is always found in ripe cheeses, some other factor must be responsible for the production of this compound. It may owe its origin to microorganisms not yet discovered.

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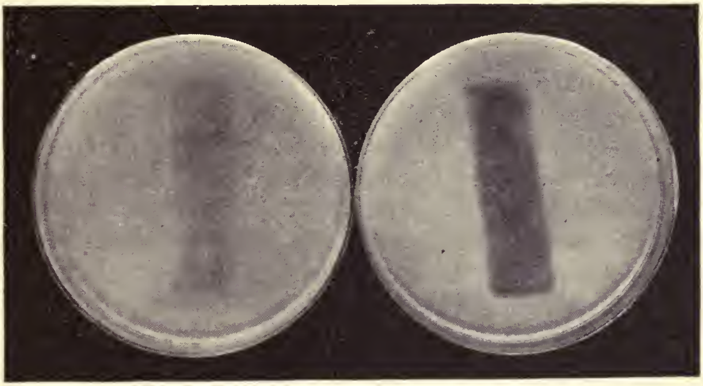
* Cheddar cheese.

Prevention of Putrefaction.—The various stages in the decomposition of milk have been outlined in a previous chapter. Briefly they are as follows: The first evident change is the curdling due to the acid-forming bacteria. Succeeding this, the acid, semi-solid mass or curd is a favorable substratum for the characteristic mold of milk, *Oidium lactis*, which soon forms a white, velvet-like layer over the surface of the milk. Like other molds, this form can use acids as a source of energy. The acid is then oxidized to carbon dioxide and water, and thus the reaction of the milk is slowly changed until a point is reached which allows the putrefactive bacteria, that have remained dormant during the period of unfavorable environment, to develop. The curd is accordingly peptonized and putrefaction occurs. If the acid reaction is maintained through the prevention of mold growth, the milk will be preserved from



A *B*

FIG. 148.—Proteolytic action of *rennet extract* in the absence and in the presence of acid-forming bacteria. *A*, sterile milk agar; a strip of filter-paper treated with *rennet* was allowed to remain on the medium for one hour at 37°. No digestion of the casein. *B*, milk agar inoculated with *Bact. lactis acidi;* incubated for twenty-four hours at 37°, then treated as *A*. True digestion of the casein is indicated by the clearing. (*Original.*)

the attacks of putrefactive organisms and will remain unchanged for an unlimited time.

The second rôle of the acid-forming bacteria in cheese is to protect it against the putrefactive organisms that are constantly present in milk and hence in cheese. The acid reaction of the cheese, due to the persistence of lactic acid, or to the formation of volatile acids after the initial fermentation, is sufficient to prevent the growth of the putrefactive bacteria within the cheese. If the cheese is made from milk which contains no acid-forming bacteria and few putrefactive ones, or if the sugar is removed from the curd by washing it with water, the cheese will not ripen since there is no acid to activate the pepsin; the curd will remain in much the same condition as when it was removed from the press. Cheese made from milk containing no acid-forming bacteria but many putrefactive bacteria is likely to undergo putrefaction, since the latter class of organisms finds conditions for growth in the absence of an acid reaction. Such a condition is rarely noted in a hard cheese under normal conditions, but may be produced experimentally. The biological acid may be replaced by other acids added to the curd in appropriate amounts, since these will activate the pepsin and protect the cheese against the attacks of putrefactive bacteria; but it is not certain that the cheese will develop a normal flavor when lactic acid is replaced by mineral acids.

Other Groups of Bacteria in Cheese.—It has been shown at the Wisconsin Experiment Station that other groups of bacteria are constantly present in Cheddar cheese. The development of certain members of the *Bact. bulgaricum* group occurs somewhat later than that of the *Bact. lactis acidi* group. It occurs largely after the sugar has disappeared. Their numbers approximate those of the *Bact. lactis acidi* group. Coccus forms are also found in great numbers in the cheese. It is probable that these two groups may be responsible for the ammonia production, since typical cultures of both groups are able to produce small amounts of ammonia in sterile milk.

Flavor Production in Cheese.—The factors that have been discussed are undoubtedly the most important ones concerned in the proteolysis of the curd, and are thus the factors concerned in the changes of texture, solubility and digestibility. The flavor, which develops during the ripening process, has been regarded as due to the proteolysis of the paracasein. A thoroughly ripened cheese contains a large amount of ammonia and related compounds. It was thus natural to consider the flavor due to these simple products of protein degradation. More recently it has been discovered that the intensity of flavor does not necessarily correspond to the content of the cheese in these products; indeed a cheese may have a high content of nitrogen as ammonia and yet be low in flavor.

The Wisconsin Experiment Station has found that the volatile fatty acids of Cheddar cheese increase as the ripening progresses. In the following table are given the data obtained from the detailed study of a normal Cheddar cheese.

ACIDS IN 100 G. OF DRY MATTER

	c.c. of N/10 alkali neutralized				
	3 days	42 days	3 months	5½ months	10 months
Lactic acid	84.09	90.28	124.00	103.70	74.10
Acetic acid	11.59	29.44	24.25	25.86	12.64
Propionic acid	0.41	2.15	3.42	1.07	2.63
Butyric acid	0.73	2.17	3.50	4.82	5.45
Caproic acid	0.00	0.36	0.96	1.25	2.23

It will be noted that the content of the higher volatile acids, those especially marked in odor, continually increases. It is possible to separate other volatile compounds found in cheese from the volatile fatty acids by distilling with steam, neutralizing the distillate with an alkali and redistilling; the second distillate will contain the alcohols and esters present in the cheese. Such a distillate prepared from Cheddar cheese is found to possess the characteristic aroma of the cheese in question. The esters it contains are largely those of ethyl alcohol. The acid-forming bacteria, as stated previously, produce varying amounts of volatile acids and slight amounts of alcohols and esters. It is likely that the larger part of the volatile compounds found in the ripening cheese is formed in fermentations which take place subsequent to the initial fermentation of the lactose. The flavor of Cheddar cheese, therefore, owes its origin very probably to the fermentation of the lactose, and to the further change which the products of the initial fermentation undergo under the influence of biological factors yet unknown. That some biological factor is concerned in the production of flavor in Cheddar cheese is indicated by the fact that if changes are made in the methods of manufacture, changes in flavor are likely to result. If the salt is omitted, the typical flavor does not appear. This can be explained only by the action of the salt on certain types of bacteria, which, in its absence, are able to grow and produce compounds that are not found in a normal cheese. Apparently the methods of manufacture establish a certain equilibrium in the bacterial life which results in the production of definite substances in amounts varying within certain limits. If any condition is varied too widely, a deviation in the microbial balance is produced and the products formed in the cheeses are changed in kind or in amounts, either of which may result in a change of flavor.

Abnormal Cheeses

The development of a normal texture and flavor in Cheddar cheese is largely dependent on the presence of definite types of bacteria. If these are replaced, wholly or in part, by other kinds, the product is likely to suffer in texture, flavor or both. As has been emphasized previously, the bacterial content of the milk is of the greatest importance in cheese, since the organisms in the milk pass into the cheese and there produce the same products as they would have done in the uncurdled milk. All abnormalities of the cheese so far as they are occasioned by bacteria are due to the abnormal flora of the milk. To the raw material the maker must direct his attention if a fine product is to be prepared.

Gassy Cheese.—The most frequent trouble encountered and the one of greatest economic importance is the fermentation caused by organisms belonging largely to the *B. coli-aerogenes* group. It has been seen that these produce in milk gases, such as carbon dioxide and hydrogen, and offensive smelling and tasting compounds. In cheese similar compounds are formed by these organisms; the gas causes the more or less abundant formation of holes which give to the cheese judge an indication of what may be expected with reference to flavor. All milk contains some of the gas-forming organisms, but it is only when they are numerous that marked injury is done.

Gassy cheese may also be due to the presence of lactose-fermenting yeasts which are usually found in milk in such small numbers that they cannot compete with the lactic bacteria in the fermentation of the sugar in the cheese. At times the number may be increased to such an extent that the major part of the sugar is fermented by them, alcohol and carbon dioxide being produced. An outbreak of gassy Swiss cheese was found by Russell and Hastings to be due to such yeasts that had gained entrance to the milk from the whey-barrels because of careless washing of the milk cans. The cheese makers of the country are realizing the importance of the contamination of the milk from the transportation of whey and milk in the same can. The most practical means of preventing trouble from this practice is to heat the whey to 68° as it passes from the cheese vat to the storage tank. This temperature destroys the harmful microörganisms, and if the storage tank is kept in a sanitary condition, the whey is sweet when returned to the farm in the milk can. It has been demonstrated that such a treatment of the whey results in a marked improvement in the quality of the product.

Miscellaneous Abnormalities of Cheese.—*Bitter cheese* is produced by bacteria that form a bitter principle. An outbreak of bitter cheese investigated by Hastings was found to be due to the replacement of the normal acid-forming flora by a lactic organism which produced such an intense bitterness as to mask the acid taste in the milk and cheese.

Colored cheese is produced by chromogenic bacteria. In case the colonies are not numerous and the pigment formed is not soluble in any of the constituents of the cheese, the color will appear as colored specks, such as the rusty spot investigated by Connel and Harding, which is due to red forms of *B. rudensis.* If the colonies are very numerous, or if the pigment is soluble, the curd may be uniformly colored.

Putrid cheese is caused by the absence of sufficient acidity to hold the putrefactive bacteria in check. This trouble is rare in cheddar cheese, since such cheese is made from ripened milk. Fruity flavors are asserted to be due to yeasts which form fruit esters.

Moldy Cheese.—In the moist air of the curing-room the cheese forms an excellent substratum for the growth of common molds whose pigmented spores discolor the surface of the cheese and thus impair its value because of the appearance rather than by any effect in the flavor. Cheddar cheese is protected effectively from molds by dipping the cheese, when two or three days old, in melted paraffin which excludes the air from the spores on the surface of the cheese.

SPECIFIC KINDS OF CHEESE

The greater part of the cheese made in this country is of the cheddar type. Other kinds of cheese are made, however, to a considerable extent. Due to the fact that these cheeses are manufactured primarily in other countries, the phrase "foreign cheese" is often applied in contradistinction to the domestic or cheddar cheese. It has not been possible to manufacture in this country all the commercially important varieties of foreign cheese. Up to the present the manufacture of certain types has been successful only in the localities in which they originated. Variations in climate or in other conditions in other localities cause deviations from the normal ripening. It is probable that when the knowledge of the essential biological factors concerned in the ripening of each type is complete, it will be possible to make any variety at will. Considerable progress has already been made in this country in the making of certain varieties of foreign cheese through the work of the Dairy Division of the United States Department of Agriculture.

CHEDDAR CHEESE.—*Cheddar cheese,* treated in much detail in the foregoing considerations because it is the most important American cheese, is made in England and her colonies and in the United States It appears in many varieties and by the American consumer is often called American cheese in distinction from the foreign cheeses. This distinction is not wholly applicable at the present time.

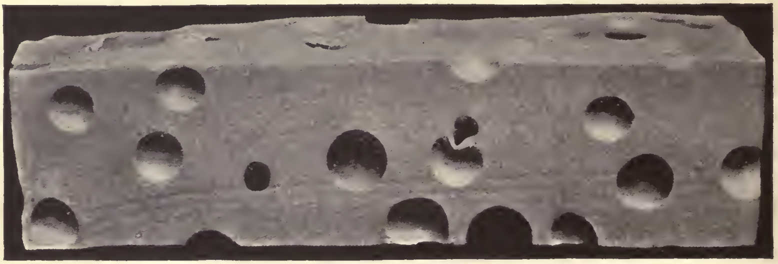


FIG. 149.—Typical development of "eyes" in Swiss cheese. (*Original.*)

EMMENTHALER CHEESE.—*Swiss or Emmenthaler cheese* originated in Switzerland, but is now made in various other countries. A large amount is made in Wisconsin, Ohio and New York. It is characterized by its sweetish flavor and by the so-called "eyes," which are holes formed by gas, produced in a fermentation that occurs subsequent to the fermentation of the lactose (Fig. 149). The number of eyes is not large and they are evenly distributed throughout the mass of the cheese except near the surface.

The cheese is made from as fresh milk as it is possible to secure. The rennet used is prepared by placing a piece of the dried rennet in whey and incubating the same for twenty-four to thirty-six hours at 30°. This is employed in place of the commercial extract used by the Cheddar maker. It serves not only to curdle the milk, but adds to it a large number of acid-forming bacteria that have grown in the rennet solution during the period of incubation. The number is not, however, sufficient to cause any development of acid during the making process which differs from the preparation of Cheddar cheese in the method of firming the curd. This is accomplished by heating the curd to 52° to 60°, and by cutting it into pieces scarcely larger than grains of wheat. The salt is applied to the exterior of the cheese by immersion in brine for one to four days and by sprinkling salt on the surface.

The fermentation of the lactose proceeds rapidly during the pressing and subsequent thereto, so that within a few days the sugar has disappeared. The lack of the development of acid during the making probably results in a somewhat different relation between the acid and protein from that existing in a Cheddar cheese, which, together with the absence of salt gives a somewhat different environment, thus making possible the development of a different flora. There is no ground for believing that the agents concerned in the proteolytic changes are other than those that function in Cheddar cheese. The flavor must, however, be due to other factors; this is indicated by the fact that if the milk is ripened as in the Cheddar process, or if salt is added to the curd the flavor will approximate the Cheddar flavor. The formation of the eyes is inhibited by salt, as is indicated by their relative scarcity in the outer layers of the cheese. Jensen has shown that the eyes are due to the fermentation of lactates with the formation of propionic and acetic acids, and carbon dioxide. The causal organism is found in the milk and the whey rennet. It is believed that lactic bacteria of the *Bact. bulgaricum* group are important factors in the ripening of Swiss cheese. They are present in large numbers in the rennet and cheese. Mixed cultures of an organism of this group and a mycoderma are used with success in Switzerland for the inoculation of the whey in which the rennet is to be soaked. The exact rôle of this form of lactic organism is not known; de Freudenreich considered them to be concerned in the proteolysis of the paracasein, since he had found that the content of sterile milk in soluble nitrogen increased when inoculated with the organism. It has been found possible by Rogers and his associates to employ commercial rennet and pure cultures of organisms of the *Bact. bulgaricum* group in the making of Swiss cheese. It has also been found advantageous to add to the milk a small amount of a culture of the eye-forming organism. It seems probable that the use of these pure cultures will result in a greater uniformity of the product than it has been possible to attain by following the empirical methods commonly used. It is probable that the formation of eyes and the flavoring compounds are due, in part at least, to the same factors.

In the other kinds of cheeses to be described, the rôle of the acid-forming bacteria is similar, if not identical, to their rôle in Cheddar cheese, *i.e.*, in activating the pepsin of the rennet and in preventing the growth of putrefactive bacteria. The factors concerned in flavor development are different.

ROQUEFORT CHEESE.—This cheese, which is prepared almost exclusively in the Department of Aveyron in southern France, is made from sheep's milk. Its most striking characteristic is the marbled or mottled appearance of the interior, due to the growth of a mold, *Penicillium roqueforti*, Thom. The curd is inoculated with the mold, when it is placed in the press, by sprinkling the curd with bread crumbs on which the mold has grown. The growth and sporulation of the mold in the interior of the cheese are favored by piercing it with small needles, thus admitting air. The characteristic flavor is due, partially at least, to the mold.

This cheese is cured in caves having a temperature below 15°. The fermentative processes are apparently closely dependent on the moisture and temperature conditions of the curing room. This emphasizes the importance of biological factors in the ripening process.

GORGONZOLA CHEESE, prepared in Italy from cow's milk, and STILTON CHEESE, made in England are similar to Roquefort in appearance and contain the same mold—*Penicillium roqueforti.*

CAMEMBERT CHEESE.—The soft cheeses are best represented by this important French cheese made from cow's milk by the addition of rennet. The milk is ripened to an acidity of 0.20 to 0.25 per cent before the addition of the rennet. The curd, which thus contains many acid-forming bacteria, is neither cut nor heated so that the maximum amount of whey is retained. The curd is placed in small hoops and allowed to drain without pressure. Salt is applied to the surface of the cheese.

The milk sugar is rapidly fermented and the resulting acidity is high, for the cheese contains 60 to 70 per cent of moisture when fresh and 50 per cent when ready for consumption. The high moisture content of the cheese and the humidity and temperature conditions of the curing room favor the rapid development of microörganisms on the surface of the cheese. Both molds and bacteria thrive under the influence of these favorable conditions, changing the cheese to a soft, smooth and butter-like mass, while a characteristic flavor is developed.

In three or four days the cheese becomes covered with the growth of *Oidium lactis;* the characteristic mold of Camembert cheese, *Penicillium camemberti*, appears later, within five to six days. These molds reduce the acidity of the curd, and through the enzymes, which they produce and which gradually diffuse into the cheese, proteolyze the curd very completely. The appearance of the cheese when cut indicates the depth to which the enzymes have penetrated; when the entire mass is acted upon, the cheese is ready for use. The reduction of the acidity by the molds exposes the cheese to the attacks of putrefactive bacteria and it soon becomes unfit for use after it is completely ripened. Several kinds of bacteria are found in the slimy surface layer, but their rôle is not known.

The development of the characteristic flavor and aroma is dependent on a certain relation between the various biological agents concerned in the ripening. This balance is dependent on very narrow conditions of temperature and humidity; slight changes in these environmental conditions favor or retard the individual types in varying degrees. If the equilibrium essential for the development of typical flavor is destroyed, this cheese fails to ripen properly and is of low value. The manufacture of Camembert cheese is a delicate problem in the ecology of microörganisms, and because of this fact the manufacture is attended with greater difficulties than is the case with most types of hard cheese.

CHAPTER IV*

RELATION OF MICROORGANISMS TO SOME SPECIAL DAIRY PRODUCTS

GENERAL

There is a number of special dairy products which do not normally come into a discussion of market milk, butter or cheese, but which are of considerable importance. A book of this sort would not be complete without a discussion of some of these products from the bacteriological standpoint. Some of these special products have been developed as commercial enterprises and the processes of manufacture have been zealously guarded as trade secrets. The result is that there is very little available data on the manufacture of these products and very little authoritative knowledge about their bacteriological condition. It is, therefore, difficult to give a full discussion of the microbiology of these products. A few of the more important ones will be discussed, however.

CONDENSED MILK

There are at least three quite distinct kinds of condensed milk made under conditions which result in an entirely different bacteriological condition in the finished product. These different products must, therefore, be considered separately. Condensed milk means simply milk from which a large part of the water has been removed, thus decreasing its bulk, the purpose being to lessen the cost of transportation and to increase the keeping quality of the product. Water is removed from milk by some process of heating, either with or without vacuum, the heating process being more or less equivalent to pasteurization.

SWEETENED CONDENSED MILK.—This product is made by reducing cow's milk at the ratio of two and one-half to two and three-fourths parts of fresh milk to one part condensed milk, by means of heat and the addition of cane sugar. It is then put up in sealed cans.

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* Prepared by W. A. Stocking.

It is not intended to be sterile. The degree of heat to which it is subjected is not sufficient to kill all of the microörganisms present and it is also subject to infection after the condensing is completed. Cane sugar is added to the milk, making the final product contain about 25 per cent of water, 35 per cent milk solids and 40 per cent cane sugar. The low percentage of moisture together with the added sugar tends to preserve this product against the action of microörganisms. There may be some bacterial growth, the rapidity depending upon the temperature at which the product is kept, but it is usually slow and milk prepared in this way will keep for a considerable time without undergoing marked bacterial changes. When gas producing bacteria exist in the milk and the cans containing the organisms are allowed to remain at warm temperatures, they will develop in spite of the large percentage of sugar, producing sufficient amounts of gas to cause the ends of the cans to bulge out. Such cans are known commercially as "swell-heads."

Unsweetened Condensed or Evaporated Milk.—In this form of condensed milk approximately the same amount of moisture is removed as in the sweetened product but no sugar is added. The decreased amount of moisture tends to prevent the rapid growth of bacteria, but this is not enough to guarantee the keeping quality of the product. After the milk is condensed it is put into cans, hermetically sealed, and then placed in steam sterilizers and subjected to temperatures somewhat above the boiling-point. In this way the milk is heated a sufficient length of time to insure perfect sterilization of the contents of the cans. If this process is properly done, the finished product contains no living microörganisms and from the bacteriological standpoint the milk should keep indefinitely.

Sometimes the unsweetened product is sold in bulk in cans. In this case it is subject to more or less contamination after heating and is not sterile, but because of the small amount of moisture and the concentration of the milk solids, the bacteria do not develop rapidly and if kept at a cool temperature, the milk will keep for some time without undergoing appreciable biological fermentations.

Concentrated Milk.—There is now on the market a form of condensed milk prepared by a different process, which is commonly known as concentrated milk. By this method the water in the milk is removed by means of dry air instead of by vacuum as is the case with condensed milk. The milk is first heated and then air under pressure is forced through it. By this process the milk is heated to a temperature of 60° (140°F.), and this temperature maintained for two hours, during which time air is forced through the milk causing violent agitation and the removal of the moisture. At the end of this time the milk is reduced to one-fourth its original volume.* The result of this process is a pasteurized milk, with a marked reduction of the original germ content. Investigations by Conn failed to show the presence of *B. coli* in milk prepared by this process. The reduction in the bacterial content of the milk is similar to that secured by other methods of pasteurization. No additional sugar is added to this milk so the product is, therefore, a pasteurized milk containing a small amount of moisture. Because of the small amount of moisture and the concentration of the milk solids, the bacteria which survive the heating process do not grow rapidly at low temperatures. The following figures will serve to illustrate the effect of this process upon the bacterial content of milk:

Bacterial count per c.c. in original milk	Bacterial count per c.c. in finished product
1,250,000	15,000
3,000,000	21,000
518,000	26,000
894,000	9,950
796,000	10,000
150,000	5,000

The rate at which the bacteria develop in this milk is shown by the following counts:

Number of sample	Bacterial count per c.c.		
	2 days old	4 days old	6 days old
1	18,000	39,000	46,000
2	55,000	28,000	39,000
3	3,500	11,000	10,000
4	4,400	5,270	4,630

The lack of moisture and concentration of milk solids prevents the rapid growth of these organisms so that bacterial changes do not take place as rapidly as in ordinarily pasteurized milk retaining its normal moisture.

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* Datafurnished by H. W. Conn.

POWDERED MILK.—This product is produced by carrying the extraction of the water farther than in the case of the condensed milks. The water is removed to a point where the milk solids can be reduced to a powdered form. This product contains the original milk solids with a very small percentage of moisture usually not more than 2½ per cent. There are several forms of powdered milk now on the market produced by somewhat different methods. In some cases the moisture is removed from the milk by its being exposed to a heated surface in a thin layer. Sometimes the drying is done in vacuum. The resulting product is dry and can be ground to the form of flour.

Another process is to remove the moisture by spraying the milk by means of an atomizer into the top of a hot chamber, the moisture being removed while the fine particles of milk are falling to the floor. By this process the product accumulates on the floor as a very dry flour and does not require any grinding. In the first process the heat is sufficient to pasteurize the milk while in the latter process it is pasteurized before being subjected to the drying process. The powdered milks do not claim to be sterile but are preserved against subsequent action of microorganisms because of the very low percentage of moisture which they contain. It is probable that there is no appreciable increase in the number of bacteria in milk flour and the product will keep for a long time without undergoing bacterial fermentations.

CANNED BUTTER AND CHEESE

Some effort has been made to put up butter and cheese in hermetically sealed cans, the purpose being to increase the keeping qualities of the products and influence the flavor by controlling the development of the aerobic bacteria. Only a limited amount of bacteriological work has been done on these canned products and the biological changes which take place in them are not very well known.

SPECIAL MILK DRINKS MADE BY THE ACTION OF MICROÖRGANISMS

From time immemorial fermented or sour milk has been used as an article of food. We are told that Abraham* placed "curdled milk" before his guests and that Moses told the Israelites that curdled milk was one of the blessings which Jehovah had given to his chosen people.* History also tells us that the wandering tribes of Arabia used fermented milk as a beverage. For centuries many of the tribes of eastern Europe and western and middle Asia and parts of Africa have used sour milk for food. Each of these regions appears to have had its own particular milk beverage resulting from the particular bacterial flora of the region.

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* Genesis 18:8. The Hebrew word "hemah" translated in the English authorized version of the Bible "butter" means "curdled milk." Century Bible, Vol. Judges and Ruth, p. 72.

The sour milk products which are now on the market under a variety of names have been derived from these original sour-milk drinks of antiquity. Fermented milk beverages have become very popular during the last few years among all the civilized peoples, partly because they make a pleasant drink but more especially because of their supposed therapeutic value.†

KUMYSS (KOUMISS, KUMISS, ETC.).—Kumyss derives its name from the Kumanes, a Russian tribe which lived along the river Kuma. This drink was prepared from mare's milk by placing it in a leather bag and adding a small amount of old kumyss as a starter.‡ In this country kumyss is made from cow's milk. This product is now placed upon the market by a number of companies who keep their methods, so far as possible, from their rivals by maintaining strict secrecy in regard to the methods of preparation. Dr. Piffard‖ who has done special work on this product states that kumyss is fermented by the action of yeasts and lactic bacteria. This fermentation produces approximately 1 per cent of alcohol and about 0.75 per cent of acid. Kumyss is strongly effervescent. The lactic organisms used in the preparation of this material appear to be a strain of the common *Bact. lactis acidi.*

Kumyss can be easily prepared in the household by the addition of cane sugar and baker's yeast to fresh, warm milk which should be kept at a temperature of about 38° (100°F.) until gas begins to form. It should then be bottled and be kept at a cool temperature. In one or two days a slight amount of alcohol will be formed and a sufficient amount of carbon dioxide to cause marked effervescence.

KEFIR (KEFYR, KEPHIR, KEFR, ETC.).—Kefir was originally made and used by the inhabitants of the Caucasus Mountains. It was made from the milk of goats, sheep or cows and was fermented by the addition of "kefir grains" to the milk. The origin of these kefir grains is unknown but the natives believe that they were the gift of Mahomet and are carefully preserved by them.

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* Deut. 32:14.

† Metchnikoff's Prolongation of Life.

‡ Milch Zeitung, September, 1889.

‖ New York Medical Journal, January 4, 1908.

Kefir was prepared by the natives by placing milk in a goat-skin bag and shaking it at intervals until it began to ferment. The kefir grains were then removed, dried and preserved for future use. The fermented kefir was also used as a starter for inoculating new lots. This beverage is now commonly made by more scientific methods.* The principal points to be observed in the preparation of kefir are cleanliness and proper temperature for fermentation and the regulation of the fermentation so that not the acid but the alcoholic fermentation will prevail.† Good kefir should be highly effervescent, should be free from lumps and contain about 1 per cent. of acid but show no marked tendency to whey off. According to Kern, kefir is fermented by a mixed culture of yeasts and bacteria in symbiosis. He found but one form of bacteria present in the cultures he studied. De Freudenreich‡ made an extended study of the flora of kefir. He prepared the kefir from the kefir grains and isolated the organisms present, putting these organisms together in different combinations in order to determine which were necessary for the proper fermentation of the kefir. He found the kefir contained four different organisms:

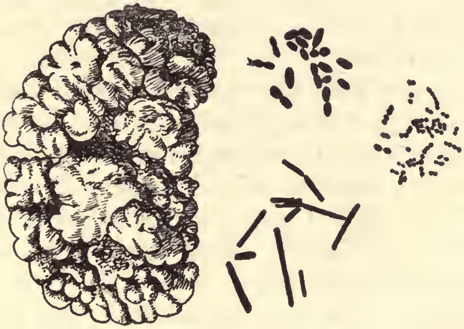

FIG. 150.—A large-sized kefir grain and the three species of bacteria of which it is composed. (*From Conn, after de Freudenreich.*)

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* Milch Zeitung, 1885, p. 209.

† F. Stohman, Milch and Molkerei Products, p. 1006 to 1013.

‡ Centr. für Bakt. Abt. 2, Vol. 3, 1897.

yeasts, streptococci, micrococci, and bacilli. The yeasts and streptococci were plated in gelatin without difficulty but it was very difficult to grow the other two organisms present on any artificial media. He concluded that the yeasts present in kefir are not identical with the species commonly used in making beer and named it *Saccharomyces kefir*. The streptococcus curdled milk in less than forty-eight hours at a temperature of 37° but the micrococcus did not curdle milk at all, although it produced a considerable amount of acid.

De Freudenreich changed the name of the bacillus from *Dispora caucasica*, given it by Kern to *B. caucasicus*, because it did not produce spores as Kern supposed. He also found that this organism would not grow at all on media without sugar, very slightly on milk, serum, agar, and best of all in milk, in which it produces both gas and acid without curdling the milk. This organism is 5μ or 6μ in length by 1μ in width, is slightly motile and retains Gram's stain. It has a thermal death-point of 55° for five minutes.

The preparation of good kefir seems to depend upon the combined action of the four types of organisms described. Kefir is sometimes prepared without the use of the kefir grains* by placing milk in bottles to which is added a small amount of compressed yeast and sucrose. The bottles are then held at a temperature of 10° to 15° about fifteen hours and shaken occasionally. Kefir prepared in this way gives an effervescent mild flavored drink.

LEBEN.—For centuries the Egyptians have used a fermented milk drink known as *leben* or *leben raib*. This was prepared from the milk of cows, buffaloes, and goats. In general it resembles the other fermented milk drinks in the fact that the fermentation is produced by yeasts and a variety of other microörganisms working together. At least one yeast and three species of bacteria seem to be normal to this product. A fermented milk drink very similar to leben is also used in Algeria. The exact action of each microörganism concerned in the fermentation of this product is not certain, but it is probable that all of the species are essential for the production of the particular flavor and consistency of the fermented product. It is claimed that the fermentation that takes place in the milk renders it more digestible than raw milk. For this reason it is recommended for the use of invalids and persons having weak digestion.

YAHOURTH OR MATZOON (YOGURT, YAHOURD, MADZOON, ETC.).—A fermented milk drink known by one of the above names has been used by the Bulgarian tribes for a long time. Some years ago it was studied and brought to public notice by the investigations and writings of Metchnikoff,* who was struck by the longevity of the tribes using this product as a part of their regular diet. As a result of his investigations, Metchnikoff has advanced his theory regarding the antiseptic power of certain strains of lactic bacteria in the digestive tract. His theory is that certain species or types of bacteria which are able to resist the action of the stomach and can, therefore, pass through into the intestines have the power of checking the growth of the putrefactive bacteria existing there and thereby prevent the production and absorption of bacterial toxins which cause autointoxication. As a result of his experiments, Metchnikoff came to the conclusion that the acid organism (*Bact. bulgaricum*)† found in yahourth was able to establish itself in the intestinal tract and produce enough lactic acid to hold in check the putrefactive processes which otherwise exist there.

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* Milch Zeitung, 1888, p. 393.

Yahourth is made by the Bulgarians in skin bags in the same way that the Russian tribes prepare kumyss. It is similar to the other fermented drinks already described in the fact that it is produced by a mixed flora of microörganisms. At least one yeast is present and two or more species of bacilli capable of producing lactic acid in relatively large amounts. These two organisms are known as *Bact. bulgaricum* and *Bacillus paralacticus*. Herter states that *Bact. bulgaricum* is 4μ to 6μ in length by 1μ in width and grows singly or in pairs and occasionally in chains. It stains with ordinary aniline dyes and by Gram's method. It grows with difficulty on ordinary laboratory media and is therefore hard to obtain in pure cultures. These organisms produce a much higher percentage of acid than the common *Bact. lactis acidi* and also grow at a much higher temperature.

This makes it possible to secure it in practically pure cultures by growing it in milk at a high temperature. Grown in pure cultures, the *Bact. bulgaricum* will produce from 1 to 2 or more per cent of acidity. It grows well at temperatures between 37° and 40° and even higher. Recently a number of fermented milk drinks have been put upon the market which have evidently been derived from the yahourth. These are sold under such trade names as *zoolak*, *vitalac*, *yogurt*, *fermenlactyl*, etc. The flora of these preparations appears to be practically the same as that of the original yahourth.

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* El., Metchnikoff, Prolongation of Life.

† Hastings has found this organism also common in cow's milk in this country.

All of the fermented milk drinks thus far discussed are similar in that each contains a variety of microörganisms, made up of at least one species of yeast with one or more species of bacteria, capable of producing greater or less amounts of acid. In some, as in the case of kefir, the yeast fermentation is allowed to predominate, while in others, like yahourth, the action of the yeasts is held in check by the rapid development of the acid by the *Bact. bulgaricum.* All of these drinks are commonly recommended by physicians because of their beneficial effect upon the digestive tract.

ARTIFICIAL BUTTERMILK.—In recent years there has developed an important industry in the manufacture of artificial buttermilk. This is usually made by inoculating skim-milk with a culture of lactic bacteria, either *Bact. lactis acidi*, or *Bact. bulgaricum* or a combination of these two types. In making the artificial buttermilk, yeasts are not commonly added. After the milk becomes coagulated, it is then churned in order to give it a smooth, creamy consistency, after which it may be bottled and kept for some time by holding at low temperatures. Sometimes a small percentage of whole milk is added at the time of churning to make the finished product more closely resemble natural buttermilk. In making artificial buttermilk, the skim-milk is frequently pasteurized in order to get rid of the miscellaneous flora which it contains. The finished product, therefore, differs from ordinary buttermilk in the fact that it contains nearly pure cultures of the lactic organisms while the natural buttermilk will contain a more or less miscellaneous flora in which the acid organisms predominate. It is possible to obtain a more uniform product in the artificial buttermilk than in the natural product, and this is perhaps responsible for the rapid development of this industry. All of these fermented milk drinks contain enormous numbers of microörganisms, usually millions per c.c.

ICE CREAM

Ice cream is one of the important manufactured dairy products and its use seems to be increasing steadily. Its bacterial flora varies with the materials used in its manufacture and the conditions under which it is made. It may be made from fresh cream which is only a few hours old and under good sanitary conditions. On the other hand, it may be made from cream which has been produced and handled under unsanitary conditions, kept in storage for a number of days and finally manufactured in surroundings not conducive to a low bacterial content. We are not surprised, therefore, to find a very wide variation in the germ content of ice cream, as it is placed upon the market.

An examination of 263 samples of ice cream collected in the city of Washington* showed an average germ content of over 26,600,000 per c.c. The lowest count obtained was 37,500 and the maximum was 365,000,000. A similar study of commercial ice cream in Philadelphia† showed the average bacterial content to be very high. The lowest count found was 50,000 per c.c., while the highest count was 150,200,000. In this work it was found that the bacterial content of the ice cream was in quite direct relation to the sanitary conditions of the establishment where the ice cream was manufactured. When ice cream is manufactured in a city from materials which have been shipped in from considerable distances and frequently held for several days in cold storage, it is not surprising that the germ content of the manufactured product should be high. In some establishments the cream is pasteurized before manufacturing, while in others it is used in its raw condition. Under present commercial conditions considerable amounts of condensed milk, and frequently unsalted butter, are used for ice cream making. Ellenberger‡ found that while all the ingredients used contained some bacteria by far the greater numbers were in the cream and condensed milk. This is shown by the following table giving the average plate counts from the ingredients used.

AVERAGE PLATE COUNTS OF INGREDIENTS

	Minimum count	Maximum count
Standardized cream	1,150	37,600,000
Condensed milk	31,500	59,800,000
Sugar	20	255
Gelatin	48	891
Flavoring	10	321

In normal cream held for some time, the lactic bacteria should exist in considerable numbers, but when cream is held at low temperatures these organisms do not develop rapidly. Pennington found that certain species of streptococci developed quite rapidly in cream held at refrigerator temperatures. Streptococci were found in fifty-five (80 per cent) of the sixty-eight samples examined. It was found that at refrigerator temperatures the relative growth of these organisms was greater than at higher temperatures, a fact which may account, in part at least, for the frequency with which these organisms occur in ice cream.

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* Results of work done under the direction of G. W. Stiles.

† Work done under the direction of Dr. M. E. Pennington.

‡ Cornell Memoir No. 18.

Frequently ice cream is held for a considerable time in a frozen condition before it is sold. It has generally been supposed that there is no bacterial growth in material which is held below the freezing temperature. This, however, did not seem to be the case in samples examined by the investigators already mentioned. They found in samples held about a month that there was normally a decrease in the bacterial count and also in the amount of gas production for a number of days, after which there was frequently a marked increase in the bacterial counts. These results would seem to indicate that even in the frozen condition there may be some increase in the number of bacteria present. Ellenberger* found the same general conditions as these earlier investigators as shown by the following chart.

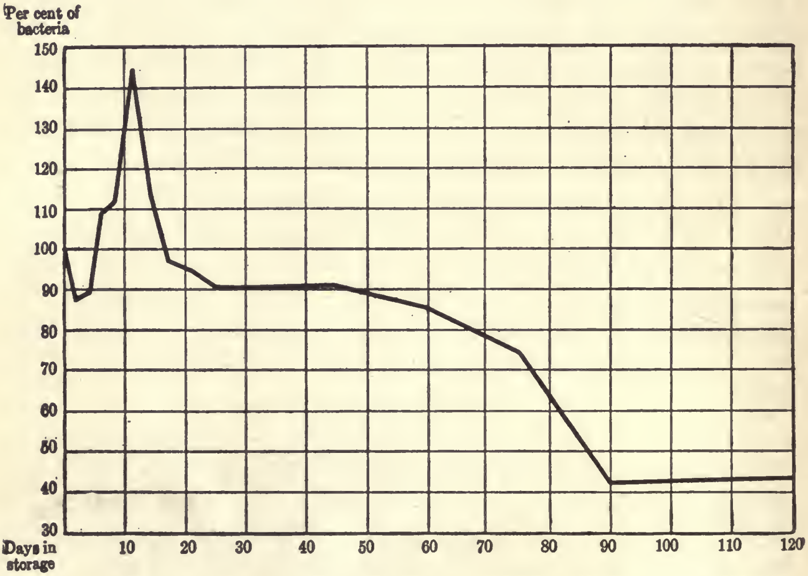

FIG. 151.—Average percentage increase and decrease of bacteria in fourteen samples of ice cream during a storage period of 120 days.

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* Cornell Memoir No. 18.

If the cream from which the ice cream is made has been produced and handled under sanitary conditions, the bacterial content should consist chiefly of organisms of the *Bact. lactis acidi* type, in which case the high count in the ice cream might not be objectionable. If, on the other hand, the cream has been held in cold storage for some time under conditions which inhibit the growth of the lactic organisms and permit the development of putrefactive types, bacterial poisons may be developed in the cream, which will be highly objectionable. There seems to be little doubt that this is the cause of the cases of ptomain poisoning, resulting from the use of ice cream. It is known that certain types of bacteria, especially those belonging to the so-called putrefactive group, are capable of developing at very low temperatures and may, therefore, produce considerable quantities of toxic products in the cream. Whether or not these products are developed before the cream is manufactured or whether they may develop in the frozen product cannot at present be stated. In general it can be said that the total bacterial count does not indicate the wholesomeness of the ice cream any more than does a similar count in buttermilk or in the commercial fermented-milk drinks. The kinds of organisms present is a far more important question from the standpoint of the wholesomeness of the ice cream. However, the results obtained by many ice-cream manufacturers have demonstrated the fact that the germ content of this product can be quite definitely controlled by the same methods of care and sanitation as are required in the handling of other forms of dairy products.

DIVISION V

MICROBIOLOGY OF FOODS

CHAPTER I*

DESICCATION, EVAPORATION, AND DRYING OF FOODS

AGENCIES THAT BRING ABOUT CHANGES IN DRIED FOODS

Food materials are derived from plant and animal tissues. The agencies which may bring about their deterioration are those initially present in the raw food, and those introduced later in the process of handling. These agencies are the *enzymes* produced by the cells constituting the food material, and the *bacteria*, *yeasts*, and *molds*, with their enzymes, which may be introduced later.

Enzymes are normally present in foodstuffs which have not been subjected to heating, since all living cells apparently contain enzymes which may remain active for a considerable length of time after the death of the cells. These enzymes are usually termed *autolytic*, that is, they digest the cells or parts of cells which produce them. They are active in bringing about deterioration of certain types of foods. These autolytic enzymes are of many kinds. Some of them attack carbohydrates, some fats, others proteins, and still others organic compounds belonging to none of these groups. They are, for example, responsible for the stiffening of muscles after death (*rigor mortis*), and later break down the tissues of the meat and bring about a so-called ripening whereby it becomes more tender. They may in some instances produce rancidity in food products by splitting of the fat. It is self-evident, therefore, that if food is to be preserved by drying, the enzymes capable of bringing about detrimental changes must either be inhibited in their action or be destroyed by heat or by some other agency. In most cases the drying of a food will remove water sufficiently to inhibit the action of the enzymes as well as to prevent the growth of microörganisms. If the process of desiccation is not properly carried out many color changes may occur which interfere with the appearance of the food product. For example, it is a common observation that the cut surface of an apple turns dark upon exposure to the air. This is due to the presence of an oxidizing enzyme. If a product resulting from the drying of apples is not to be too dark colored, either unusual care must be used in the preparation or some method of inhibiting the action of the enzyme or bleaching the product must be used. The ripening process of fruits is due to the transformation of cell contents and constituents by the enzymes contained. When this process continues too long over-ripening and spoilage occur. In other words, substances which are ill-flavored may develop in foods which are not dried rapidly enough or are dried insufficiently.

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* Prepared by R. E. Buchanan.

Bacteria are present in large numbers upon the surfaces of many raw foods. More are added during the process of handling. They probably constitute the most important single item bringing about destruction or deterioration, and whenever moisture and temperature conditions are favorable they rapidly bring about undesirable changes. Carbohydrates present in the foods are hydrolyzed and fermented, fats are frequently hydrolyzed, and proteins broken down into simpler compounds. In general they require somewhat more moisture for their development than the yeasts and the molds.

Food materials may be divided into two principal groups: those in which the desirable food constituent is in solution in water; and those in which the principal food constituent is more or less insoluble. There are many foods which combine both characteristics. Raisins, for example, contain a considerable amount of sugar in solution in the water present. Those foods which are relatively insoluble may again be divided into four groups, using the amount of water as a basis for classification. First, those in which moisture is present in appreciable quantities in the interstices, that is, those which seem *wet*. These usually furnish optimum conditions for the growth of the bacteria which multiply rapidly and spread through the medium by actual space growth, by convection currents, and by their own power of motion. Second, some foods may contain moisture sufficient for the abundant growth of bacteria but not free water which will allow of rapid distribution. In these the spread of the microörganisms must be largely by direct growth and will necessarily be slower than in the preceding. Third, the substance may be so dry that little or no growth of the organisms may take place yet there is sufficient moisture so that they remain viable for long periods of time. Fourth, the food may be so dry that only those organisms which withstand relatively complete desiccation will survive. These groups cannot be differentiated from each other wholly on the basis of the percentage of water present. The manner in which the water is held, and the substances which may be in solution in the water are also very important.

Yeasts are present upon the surfaces of many fruits. They usually require sugars for their best development, and are therefore commonly present in foods containing this substance. Yeasts will also be found upon the cut ends of twigs or grass culms where the sugary sap has oozed out. Colonies of considerable size may sometimes be seen on corn stubble during damp weather. These yeasts are commonly distributed by flies and other insects which feed upon the plant juices. The yeasts are not motile, hence their spread in any food must be as a result of direct growth or of convection currents.

Molds, like the bacteria, are ubiquitous and under proper conditions will destroy most types of food. They grow readily on solutions and on saturated substrata, but frequently are overgrown by bacteria under these conditions. For example, wet silage rots when exposed to air and supports luxuriant growth of bacteria, while drier silage becomes moldy. Unlike bacteria the molds extend through and over food when there is no visible water film. The spores are much better adapted to air dispersal than are bacterial cells, and the hyphæ penetrate more rapidly than will the bacterial colonies. In certain foods, therefore, such as meals and flours, molds are more destructive than are bacteria. Usually they will multiply with somewhat less moisture.

Factors Which Inhibit Growth of Microörganisms in Desiccated Foods

The factors which appear to be of greatest importance in inhibiting the growth of microörganisms in dried foods are: the relatively complete absence of free water, concentration of solutes, formation of water-free protective layers, and the action of heats, sunlight, sulphur dioxide, smoke, or other disinfectants or bleaching agents.

The amount of water remaining in a desiccated food is probably the most important single factor in determining its keeping qualities. In a few cases the development of microörganisms is absolutely inhibited by the absence of sufficient moisture in the food to support growth. Many foods which appear to be dry nevertheless contain an appreciable amount of moisture. The amount necessary to bring about appreciable changes or detrimental changes is sometimes not very great. For example, the amount of moisture present in raw sugar appears to constitute a real loss to the manufacturer. Some foods, such as olive oil, starches, meals, cane sugar, etc., have little or no free water. Others contain an appreciable amount of water and yet do not deteriorate, usually because the drying has resulted in a concentration of the solutes beyond the point to which the microörganisms can adapt themselves to the osmotic pressure. When it is remembered that a 50 per cent solution of cane sugar is capable of exerting a pressure of about 225 kilograms per square inch, it will be realized that considerable capacity for readjustment is necessary in the cell of any yeast or mold that can grow in such a medium.

In the process of drying, the former relationships of tissue cells and tissue constituents may be so changed that protective layers are formed. For example, in curing pork, the fat which was structurally isolated in distinct cells for the most part becomes diffused through the outer layers of the tissues and forms a water-free and water-proof exterior. The keeping quality of the dried food is sometimes in part dependent on the destruction of microörganisms by heat during the process of drying. In other cases they are exposed to the germicidal action of the direct rays of the sun, or to the action of some disinfectant or bleaching agent, such as sulphur dioxide or smoke.

Methods of Drying

The rapidity with which foods may be dried depends upon the amount of water present in the food, the texture and size of the particles, the temperature, the relative humidity of the atmosphere, and the rapidity of the current of air which carries away the moisture. The rapidity with which foods must be dried in order to remain palatable depends in part upon how subject they are to the attacks of molds, yeasts, or bacteria, in part upon the rapidity of changes brought about by autolytic enzymes, and in part upon the changes in flavor and texture which may be brought about by the application of too high a temperature.

A reduction of the water in foods below the minimum required for the growth of microörganisms is accomplished in a variety of ways. Most commonly heat is employed, either the sun's rays or from some artificial source. In localities where the humidity of the air is low, as in many of the irrigated fruit districts of the western United States, exposure to the rays of the sun is sufficient for drying. For other types of foods, and in more humid regions, artificial heat is used to reduce the relative humidity. Some foods cannot be dried at high temperatures because of their instability. They are usually dried at a low temperature and in a partial vacuum. Other foods are dried without recourse to evaporation by the use of hydraulic presses or by centrifugation, the latter in the manufacture of cane sugar. The water for the growth of microörganisms may be reduced by the addition of some crystalline substance such as sugar or salt. The usefulness of the latter method depends largely upon the creation of a concentration of solutes too great for the growth of the bacteria. At the same time a considerable proportion of the water from that part of the food into which the solutes will not penetrate is extracted by osmosis.

Many food products do not require any special drying as they naturally contain little moisture. Such are the grains and the products manufactured from them, as flour. The drying in this instance has occurred during and immediately following the ripening process of the grain. When for any reason this does not occur the grain will mold. It has been found necessary in many instances to kiln-dry corn. Grains, nuts, etc., are by their nature adapted to keep under normal conditions for considerable periods, although there is usually present in nuts sufficient moisture to allow of the slow action of lipolytic enzymes and consequent development of rancidity. Other foods require artificial drying. In these we have the intergrading classes which we have discussed above; those which contain a small percent of water and those which contain considerable water but a high concentration of solutes. The absolute amount of water in a food is by no means the index to the amount that is available for the growth of microörganisms. Many foods are hygroscopic. Foods having the same water content and percentage of solutes will behave very differently with reference to delivering up the water to an organism present.

The effect of the concentration of solutes by drying is perhaps the most important factor in the preservation of foods. These substances dissolved in the water may be actually antiseptic when concentrated, as the acids of the juices of certain fruits. More often the sugars reach a concentration so great as to prevent growth by plasmolyzing the cell contents of the organism. For every organism there is a maximum concentration reached sooner or later beyond which growth is impossible.

Dried foods may be divided into three groups using the relative abundance of carbohydrates, fats, and proteins, as the basis of classification.

Carbohydrate foods are usually preserved by drying. Many, such as grains and nuts and the flours and meals prepared from them, do not require artificial heating. They are, however, somewhat hygroscopic and in damp climates enough moisture is taken up to allow the growth of injurious molds and bacteria. Still other carbohydrate food stuffs require more or less care in the drying or curing, such as hay and fodder in general. These are usually dried by exposure to the air and sun until most of the water has been evaporated. Fodder that has become moldy through the presence of too much moisture is the cause of trouble in horses and less frequently in cattle. Many deaths due to the so-called cerebro-spinal meningitis in the horse are frequently due to the consumption of moldy hay. In localities where the air is too moist or rains so frequent as to make it difficult to dry hay, curing is effected by a process of self-fermentation. The hay is piled in a mass while still green and undergoes a process of heating. The temperature rises to about 70°. The cause of this rise is somewhat uncertain but is probably due to the combined action of enzymes and microörganisms. Just how much of the keeping quality is due to the heating, how much to the loss of water, and how much to the accumulation of products of fermentation is uncertain. In other cases the heated hay is spread out and quickly dried sufficiently so that it may be stored. A certain small percentage of the nutriment in the hay is necessarily lost in the development of the heat.

Many vegetables in desiccated form can now be bought upon the market, and have been prepared in recent years in large quantities for household use by the housewife. Fruits are also quite generally preserved by drying. In many instances, as in peaches, apples, and berries, it is probable that enough moisture is removed to prevent organisms from growing, but in many other cases, as in the preparation of currants and raisins, the concentration of sugar and other solutes is the controlling factor. Frequently as much as 30 per cent of the dried fruit is water. Fruit drying is often accomplished by heating in the sun's rays, in other cases artificial heat and even hydraulic pressure are used.

Many manufactured products, particularly bakers' goods such as crackers, biscuits, dried yeast cakes, etc., are preserved by the elimination of water.

Macaroni and *vermicelli* are prepared by forcing a thick paste of especially prepared flour and water through openings of different sizes. The product is then dried in the air until it is brittle and may then be kept indefinitely.

Copra, one of the principal exports of certain of the islands of the Pacific and Indian oceans, is prepared by cutting the meat of the cocoanut into pieces and drying in the sun. From this copra much of our desiccated and powdered cocoanut is prepared, and from it is pressed the cocoanut oil which finds so many uses in manufacture.

Syrups, *molasses*, *jellies*, *jams*, and many other carbohydrate foods are preserved through the concentration of solutes. Many of these are partially sterilized by the heat used in the process of manufacturing. There is usually plenty of opportunity for subsequent infection. They are more frequently attacked by molds and yeasts than bacteria. An exception may be noted in *Leuconostoc mesenterioides*, a bacterium which causes considerable trouble by a gelatinous fermentation in syrups from which sugars are being manufactured.

Fatty foods frequently contain little water. Cottonseed, olive, cocoanut, and other vegetable oils, the plant and animal fats as lard, tallow and butter, are quite resistant to change by bacteria unless water is present and considerable traces of nitrogenous materials remain in them. With these foods the water is necessary for the growth of the organism and also for the action of the lipolytic enzymes which might hydrolyze fats and aid in the development of rancidity. Butter is an exception to the rule that fatty foods contain little water, as it usually has from 12 per cent to 16 per cent. When it is necessary to keep butter fat for long periods under unfavorable conditions, the water and nitrogenous material are removed and the clear fat preserved. This is the so-called *ghee* of India. Bacteria, enzymes, and a few molds have been described that attack fat. In the process of preparation or manufacture of any fat foods sufficient heat may be used to sterilize the material, and infection thereafter penetrates to the interior very slowly. This heat destroys the enzymes as well as the bacteria.

Protein foods are in large part flesh foods and flesh derivatives. Desiccation, however, is only one of the agencies acting to preserve the flesh.

Jerked meat is sometimes prepared in localities with a hot dry climate. Lean meat is cut into thin slices and exposed to the direct rays of the sun until dry. The bactericidal action of the sunlight and the rapid extraction of moisture prevents microörganisms from producing undesirable changes during the curing process.

Dried beef is lean meat which usually has been treated with certain condiments or smoked and salted and then dried.

Dried fish such as cod, mackerel, and herring, is prepared by the use of condiments, salt, and smoke in addition to the drying.

Pemmican is prepared by drying lean meat, grinding it, and mixing it with sugar and fat, dried fruits, spices, etc. It is highly nutritious, not unpalatable, and compact, and will keep for a long period. It is frequently used as a concentrated form of food by Arctic explorers, etc.

Beef extract is prepared by cooking minced beef and water in a receptacle under a slight steam pressure. The digestion is continued for several hours. The liquid is filtered off and concentrated in a partial vacuum to the desired consistency.

Gelatin is prepared by boiling bones and tendons, sometimes also horn and hide scraps and concentrating the gelatin which dissolves from these.

Somatose, sarco-peptone and related so-called predigested protein foods are mixtures of albumoses and peptones prepared by the artificial digestion and drying of proteins, usually flesh. The product is marketed as a powder.

Milk, either with or without its butter fat, is dried by being sprayed into a warm compartment from which the air is partly exhausted. It dries immediately, in the form of a very fine powder. This powder, if thoroughly dry, will keep well and is finding an extensive use. The high sugar content of this powder is instrumental in preventing the development of microörganisms.

Eggs are dried in much the same manner as milk and the product is being used extensively at the present time by bakers.

Meats are frequently preserved by a combination of drying and the action of certain antiseptics or preservatives. The salting of meat owes its effectiveness in part to the abstraction of water. In most cases, the surface of the meat and probably even the other portions are protected in large measure by the diffusion of the fat and the saturation of tissues and by the formation of water-proof fat films. The autolytic enzymes are active in the fresh meat and soon become inert upon the removal of water. The organisms responsible for decay of preserved meats and flesh foods are usually bacteria. Some of these break down the protein into simpler chemical compounds, of which a few are known to be poisonous.

CHAPTER II*

HEAT IN THE PRESERVATION OF FOOD PRODUCTS

HISTORICAL RÉSUMÉ

The principle involved in the preservation of food by heat may be said to have had its origin in the experiments of Spallanzani, who in 1765 boiled meat extract for an hour and hermetically sealed the flasks, after which treatment no change occurred in the material. An application of this principle was suggested as early as 1782 by the Swedish chemist, Scheele, who advised the exposure of vinegar in bottles to the temperature of boiling water in order to effect its preservation. Some years later the principle was applied to the conservation of food by a French confectioner, Nicholas Appert, who in 1811 published an exhaustive treatise on "The Art of Preserving Animal and Vegetable Substances." His method was to enclose the food in a glass jar which was then corked tightly, and placed in boiling water, the length of time of heating varying with the article to be treated.

In 1810 Peter Durand secured a patent from the English government for the preservation of fruits, vegetables and fish in hermetically sealed tin and glass cans. He did not claim to be the discoverer of the process, but said it had been communicated to him by a "foreigner residing abroad." Although the secret of the process was jealously guarded, the employees of different establishments became familiar with its essentials, and in this manner the industry found its way to America. One of the first to introduce the process was Ezra Daggett, who, with his son-in-law Thomas Kensett, in 1819 engaged in the manufacture of hermetically sealed goods, the principal foods packed being salmon, lobsters, and oysters. In 1820, William Underwood and Charles Mitchell, emigrant employees from a canning factory in England, opened a factory in Boston where they canned plums, quinces, cranberries and currants.

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* Prepared by S. F. Edwards.

In the earliest days of canning, glass jars were used exclusively, but were gradually abandoned as it was found that they could not readily withstand the extremes of temperature, and were expensive, bulky, and costly in transportation. In 1825, Thomas Kensett secured a patent on the use of tin cans in preserving food, and in the same year began using the process in his factory. The early manufacture of tin cans was by hand and crude, the bodies being cut with shears and the side seams made with a plumb joint (that is meeting but not overlapping), and then soldered together. Heads were made to set into the body and were soldered in place in a very crude manner. The making of 100 cans was considered a good day's work for one man. Improvements were gradually made, however, in their manufacture, until finally can making became a distinct industry and now all the parts are made and put together by machinery.

In the original Appert process, the goods were cooked in open kettles, the highest temperature obtainable by this method being the boiling point. A little later common salt was used to aid in securing a higher temperature, and this was followed later by the use of calcium chloride which made possible a temperature of 115°. In 1874, a closed kettle was invented for superheating water with steam, and this was immediately followed by another improved kettle in which dry steam was used, the principle employed being that of the modern autoclav, by which method any desired temperature may be obtained and modified to suit the requirements of different classes of food.

Economic Importance

From Standpoint of Health and Dietetics.—The value of a variety of foods, especially fruits and vegetables, is recognized by dietitians. Unfortunately, however, the season for fresh fruits and vegetables is comparatively short. Moreover, many foods grown exclusively in one section of country will not withstand shipping in a fresh condition to other sections. In spite of improved methods of refrigeration, it is not practicable to ship fresh sea foods to far inland towns, or to send some perishable products of warm climates to cold countries. The canning and preserving industry overcomes these difficulties by supplying pure, clean, wholesome fruits, vegetables, meats, and fish to any region the year round, and at prices comparatively low.

From Standpoint of Commerce.—In its commercial aspect, the importance of the industry can scarcely be estimated. Canned products make possible the carrying of larger stores of provisions by armies and navies and expeditions for exploration than would otherwise be possible. In fact, the stimulus which prompted the investigation of Appert was a prize offered by the French Navy Department for a method of preserving foods for provisioning ships more satisfactory than pickling, drying, smoking or preserving in sugar, the methods in use up to that time.

"Although the preserving industry was established in three great commercial centers in the United States as early as 1825, it did not become of much importance until the last decades of the nineteenth century. There were many hindrances to the progress of the industry, such as the secrecy observed in the process, skepticism of the public regarding the healthfulness of canned foods, the general prejudice against them, and the high cost of production. These obstacles have gradually been surmounted, and at the present time the several branches of the industry have collectively assumed large proportions.

An idea of the magnitude and importance of the industry in the United States may be gained from statistics for 1918 compiled by the National Canners' Association, and here reproduced by permission. The pack of tomatoes was 15,882,372 cases; of corn, 11,721,860 cases; and of peas, 10,898,222 cases. The total vegetable pack for 1917 other than corn, peas, and tomatoes, was 13,391,294 cases, and of fruit was 11,285,659 cases. The average case holds two dozen cans. These figures do not include the pack of oysters, meats, or fish. The total annual consumption of canned foods has been estimated at 250,000,000 cases. It is apparent from these figures that the canning and preserving industry is one of immense value, and that it constitutes a large factor in the feeding of the world.

Alteration of Food

Physical Changes.—*Appearance.*—Some physical changes attend the conservation of foods by heat, approaching more or less closely the changes incident to the ordinary preparation of fresh foods for the table. In the preserving of some fruits and vegetables the canner subjects them to a blanching or fore-cooking process which consists in submitting the product to the action of hot water for a short time. The object of blanching is first, for the purpose of removing the more or less gummy substance upon the surface of such vegetables as peas and beans; second, to make the product more or less flexible so that it may be packed without breaking, as asparagus; third, to permit the packing of a greater quantity in a can, as spinach; fourth, to force water into the product and cause it to be tender, as in beans; fifth, to secure a more uniform color, as in fruits; and sixth, for its cleansing effect. It is not a bleaching process as many infer from the term. The time used in blanching varies from one to fifty minutes, usually being between two and five minutes. The operation is of no value in reducing the time necessary to properly process canned foods.

Mechanical Disintegration.—In the case of very soft fruits or vegetables, the high temperature of processing causes a slight amount of mechanical disintegration, which is not objectionable unless excessive, as there is little deterioration in appearance and none at all in food value. In the case of meats, practically the only physical change is the shrinkage during the parboiling previous to placing in the cans.

CHEMICAL CHANGES. *Appearance.*—The chemical changes in foods preserved by heat may be considered under two heads: first, those in which the appearance is modified; and second, those in which the food itself is altered. Some change of color sometimes occurs and results from various causes. In colored vegetables, such as peas, string beans, and asparagus, a part at least of the loss of color is due to the oxidation of chlorophyll. With a few foods, iron sulphides are occasionally formed by a combination of sulphur with the iron of the container. This seldom occurs, however, and is not of great importance. Some fruits packed in glass gradually lose their color by oxidation on exposure to the light.

Chemical Change.—So far as chemical alteration of the food itself is concerned, there is little change and none other than would occur in the preparation of the food for the table. The albumins are coagulated. The fats probably remain unchanged. Of the carbohydrates, the chief action is on the sugars. The cane sugar is wholly or partly inverted by the combined action of the heat and the fruit or vegetable acids. The starch undergoes little if any cleavage, inasmuch as this change occurs only in the presence of acids and in foods with a relatively high acid content, the proportion of starch is low. The other amyloses undergo little if any change.

Palatability and Digestibility.—It is often contended that canned foods are less palatable than fresh foods of the same kind. This lack of agreeableness to the taste is, however, more seeming than real, and arises largely from the prejudice of the consumer against food conserved in tin cans rather than from any actual change. When the preserving is properly done the product should be no less attractive to the eye, no less pleasing to the palate, and of no less value from the standpoint of digestibility than the same food when served in the fresh condition.

BIOLOGICAL CHANGES. *Vital Disorganization.*—The entire industry of conservation of food by means of heat is based on a microbiological process. It is a universally recognized fact that the ordinary spoilage of food is a microbiological change, hence to protect food from spoilage consideration must be given to the microbial agents responsible for the change.

Normal Flora and Fauna.—Unlike some branches of microbiology as medical or dairy, we are unable to designate definite species as those usually identified with the spoilage of canned foods. Considering the great variety of foods preserved by heat, and the different conditions under which they are grown and secured, it naturally follows that the normal flora and fauna of food to be preserved in this manner would embrace a wide variety of species, including some higher fungi, molds, yeasts, bacteria, and low animal forms. Generally speaking, the microbial flora of fruits consists mostly of molds and yeasts, although bacterial forms may also be present. In the case of vegetables, and of fruits coming in contact with the earth, more species of bacteria are apt to be present, many of them spore formers able to withstand a high temperature. Finally, in meats and fish the living forms may include not only molds, yeasts, and bacteria, but animal forms as well, such as the organisms of tæniasis (tapeworm) and trichinosis. Weinzirl, Hunter and Thom, Sadler, and others, by investigations reported in 1918 and 1919 showed that the bacteria most often present in canned foods were of the *B. mesentericus* type, and those of the colon group. Weinzirl made bacteriological examinations of 1018 samples of canned foods including spoiled goods, experimental underprocessed samples, and market samples. "The organisms isolated comprised (*a*) yeasts, 17 cultures, (*b*) molds, 29 cultures representing 7 genera, and (*c*) bacteria, 392 cultures representing 38 species of which *B. mesentericus* (*Flugge*) was the most prevalent." Hunter and Thom examined 530 cans of salmon representing 9 brands, and found 237 unsterile cans; 224 of these contained the same organism of the *B. mesentericus* group, either in pure culture or in connection with other species. Tinned sardines show a high percentage of organisms of the colon group. As the intestines are not removed before the fish are packed, this would naturally be expected. Weinzirl concluded that food poisoning organisms such as *B. botulinus* and *B. enteritidis*, etc., are not found in commercial canned foods. Burke, however, in 1919, examined 235 cultures from a wide range of material in California, including tap water, hay, leaves, vegetables, fruits in various conditions, insects, spiders, sowbugs, snails and caterpillars, garden soil, manure from horses, hogs and chickens, and also samples from the claws and beaks and from the crop, gizzard and intestinal contents of birds. Seven cultures containing *B. botulinus* were found. Burke concluded from her research that "*B. botulinus* is widely distributed in nature; that it is present in the garden and may be on the fruits or vegetables when they are gathered." Bigelow and Estey, in a paper read before the Society of American Bacteriologists in December, 1919, emphasize the importance of further knowledge of the thermophilic organisms. "These bacteria are frequently mentioned but practically nothing has been done with them. We have quite a number of them isolated, some of them being acid formers and producing "flat sours," and others being gas forming. One of them converts starch to maltose and produces the so-called "sweet hominy." Another one turns milk bitter and has caused some spoilage in evaporated milk which was not recognized as bacterial spoilage. Some of these organisms do not appear to grow below the temperature of 42°C., and grow as high as 76°C. Others grow readily at 65°C. and as low as room temperature. We do not know how much higher or lower. These resistant spores do not appear to grow at a lower p^H value than about 4.7. They therefore are not expected to give any difficulty in processing products as acid as tomatoes or as the ordinary fruits. The lesson from the data secured is that foods should be processed at as high a temperature as possible. These resistant organisms require many hours for the destruction of their spores. The spores are not destroyed by fractional sterilization. If these spores are present and a high temperature is not used for their destruction, they will cause spoilage."

PASTEURIZATION

ECONOMIC CONSIDERATIONS.—In the preservation of food by heat, two processes are applicable, pasteurization and processing or sterilization. In pasteurization, the aim is not to effect the permanent preservation of foods or drinks by destroying all life present, but rather to destroy certain species of organisms, thus checking the natural fermentation, and effecting a temporary preservation.

The principle of pasteurization may be said to have originated in the early work of Spallanzani and Scheele, already mentioned, and was employed by Appert in his later investigations. The operation as carried out by Appert does not, however, appear to have found general application until Pasteur revived the method, and as a result of his activities in attempting to secure a general adoption of the practice to prevent the spoiling of wine, the process was named from him.

SPECIFIC APPLICATION. *Beer.*—Pasteurization is of economic importance particularly in the dairy and fermentation industries, and has perhaps had its widest application in the brewing industry. The method as stated by the Schlitz Brewing Company is as follows: "The process of pasteurization is in use with even the smallest brewers in the United States, beer being pasteurized even for local consumption. The beer is pasteurized in bottles by being subjected to a temperature of 58° to 63° for one-half hour. The entire process as practised in the large breweries requires less than an hour, and includes the warming of the cold bottles to pasteurizing temperature, the pasteurizing proper, and the cooling down to a little above room temperature. The process is a continuous one, the bottles being put into the machine at one end and taken out at the other."

Fruit Juices.—The essentials in the pasteurization of wine and fruit juices are similar to those for beer. There is, however, no universal rule of application. Details of the process must be worked out to suit the character of the different liquids under treatment.

Cream and Milk.—Pasteurization as employed in the dairy industry varies in its method of application according to the purpose for which it is used. Milk or cream as ordinarily received at creameries contains a widely variant microbial flora, many of the species exerting a greater or lesser influence in determining the flavor of the finished product.

By pasteurization of the cream, the butter-maker destroys most of the organisms present; and by the use of a culture starter of lactic acid bacteria, he is able to control the fermentation, and is assured of a uniform quality of product from day to day throughout a season. An added value of pasteurization is that all pathogenic organisms are destroyed, thus aiding in the prevention of such diseases as might be conveyed through this product. In creameries, the usual method of pasteurization is what is known as the continuous or flash process, in which the milk is subjected to a momentary heating to about 85°, the flow of milk through the pasteurizing machine being so regulated as to bring all the milk up to the desired temperature, the heating being immediately followed by rapid cooling, and subsequent addition of the lactic starter.

In the pasteurization of milk for infant feeding, a lower temperature is employed. A temperature sufficiently high to kill the organism of tuberculosis (the standard for pasteurization) by momentary heating, imparts to the milk a cooked flavor, making it less palatable, and coagulates some of the protein constituents making it less digestible. The desired end may be reached by using a lower temperature for a longer period of time, and the method generally recommended is to heat the milk to 60° to 65° for thirty minutes. This heating is sufficient to render harmless any pathogenic organisms likely to be present in the milk, without the objectionable features attendant on heating to a higher degree.

Condensed Milk.—It is commonly stated that Gail Borden invented the process for preparing condensed milk, in 1856. Previous to this, however, milk had been condensed in France, England and Germany as early as 1825 to 1835. While he cannot, therefore, be called the inventor of condensed milk, to Borden belongs the credit of having first prepared it by a rational process, and in a practicable form.

In the manufacture of condensed milk, good fresh milk is evaporated in a vacuum pan similar to those used in sugar factories, at a temperature of 40° to 50° until the volume is reduced to a little more than half, cane sugar being added so that the finished condensed milk usually contains 40 per cent cane sugar. The evaporation must be conducted with great care, otherwise the lactose crystallizes out, and this causes the product to feel "sandy" to the tongue. When the evaporation of the milk is complete, the yellowish white syrup is sealed up in tins which hold about 450 g., and this quantity is equivalent to about 1½ l. of normal milk. The addition of cane sugar acts as a preservative, and although the finished product may contain some living organisms, it is said to keep indefinitely if unopened, and will even keep for a number of days after opening. Occasional losses do occur by spoilage of the finished product, either from the growth of occasional types of bacteria tolerant of the high percentage of cane sugar, or from yeasts.

Processing and Sterilization

Economic Considerations.—For certain classes of food products, pasteurization is widely applicable, and is of great value from an economic standpoint. Preservation by pasteurization is at best, however, temporary. Bacterial spores are certain to be present on many kinds of foods, and these, unharmed by pasteurizing temperatures, develop vegetative cells, and spoilage occurs.

For permanent preservation therefore, a higher temperature and longer periods of time must be adopted. The final heating of canned foods for permanent preservation has formerly been termed *sterilization*. In the light of recent researches, this terminology must be modified.

As stated in a previous paragraph, it has been shown by several investigators that canned food may keep for a long period of time although not in a sterile condition, possibly containing viable spores of bacteria. These spores are unable to grow in the food due to the absence of oxygen. The principle employed in the canning of food to-day is the same as that of Appert over 100 years ago. Although he knew nothing of microörganisms or their relation to the spoilage of food, Appert's experiments taught him that not only must the food to be conserved be heated thoroughly, but it must also be so sealed as not to allow air to enter the container.

With the development of knowledge of microbiology it was considered that the success of the process did not depend so much upon keeping out the air, but upon keeping out organisms which might be carried in the air. This is true provided the canned food is *sterilized*. As recently proven, however, commercially canned foods may keep perfectly although not actually sterile, *provided a vacuum is established in the can* thereby preventing the viable spores of bacteria from developing.

The final heating of the food in the cans is more properly called *processing*, a term which has long been in use by the commercial canner, and under present methods of processing, the vacuum in the can appears to be as essential to the preservation of the food as the heating itself.

Conditions arising as a result of the World War have stimulated a great deal of investigation by various workers to establish the temperatures and length of time necessary for processing to secure actual sterility of food products, this condition being very properly the ultimate aim of the packer.

Specific Application.—For the preservation of fruit juices and fermented products, pasteurization is much more extensively used than processing at a higher temperature. If too high a temperature is employed for fruit juices, certain compounds of agreeable taste and aroma are destroyed, with a consequent deterioration in the flavor of the product. Fruit juices may be sterilized by heating at a low temperature for a period of time on each of several successive days.

The method of Appert has its widest application in the conservation of fruits, vegetables, meats and fish. Whatever modifications are made in the handling of the different classes of foods, the essentials are the same. The raw material, after thorough cleaning and removal of waste if any, is filled into the cans and submitted to the heating process, the degree of heat and time of processing varying with different foods. With a few exceptions, notably asparagus, vegetables are improved by heating above the boiling point. With fruits the reverse is true, the conservation of flavor being best at as low a temperature as is practicable to be employed to properly preserve them from spoilage. Briefly the methods employed in canning some foods follows:

Meat.—The canning of meat for interstate commerce is under Government supervision. No meat may be used which has not undergone inspection, the plants must comply with certain prescribed regulations, and the methods be approved. This is the only line of canning under inspection. It practically limits the canning of meat to the large slaughter houses, or to companies purchasing only inspected products, and having inspectors in their plants.

In the meat-canning industry, lean meat is largely selected for two reasons. Fat, well-finished carcasses bring a better price when offered for sale in the fresh condition; and in the second place, lean meat has a better appearance in the canned state than fat meat. The selected meat is cut into pieces of approximately from 1 to 4 pounds in weight, according to the size of the tins in which it is to be preserved. The pieces are cut as nearly as practicable the same size, not only for purposes of appearance in the cans when opened, but also that the heating process may be more uniformly carried out. If the pieces were of different sizes, the smaller ones would become thoroughly cooked and disintegrated before the larger ones were sufficiently processed.

After the pieces have been selected and dressed they are parboiled before being placed in the containers, the time ranging from eight to twenty minutes, according to the size of the pieces. The object of parboiling is to secure the shrinkage which always takes place on heating. Meats put into tins in the fresh state and processed shrink to about two-thirds of their original volume. When the meat is put directly into boiling water, there is less loss of protein than when placed in cold water and heated gradually. During parboiling, the meat loses about 1 per cent. of the protein content, about one-third of the total meat bases, and 50 per cent. of the mineral matter.

This shrinkage by parboiling tends to make a more concentrated article, thus favoring transportation, and, pound for pound, the nutritive value is not lowered. Practically, the nutritive value of a pound of properly canned beef is about one-third greater than that of 1 pound of fresh beef of the same kind. After parboiling, the meat is placed in tins and a quantity of meat jelly is added to prevent the meat from adhering to the tin in spots, and also to give it a better appearance.

Some meats are partially cured before canning, as corned beef. Sausages, and minced, devilled, and potted meats are cooked and run through meat cutters or grinders. These products are generally made from meat trimmings and pieces too small to use in the regular way. Some of these contain mixtures of meats, some cereal, and others spices. The packing of chicken, turkey, and game follows the general routine of meat packing.

Fish.—The process of fish canning does not differ materially from that of other meats. On account of its proneness to rapid decomposition, especial care must be observed that the fish are in a perfectly fresh state before canning, and that the processing be most thorough. The salmon is preëminently the sea-food in cans in this country, the value of the pack being nearly equal to all other sea-foods combined. Further, salmon is the principal fish for the preservation of which dependence is placed on sterilization alone, most fish being preserved by other methods.

Vegetables and Fruits. Corn.—Sweet corn only is used. The young tender ears of sweet corn are picked from the stalk, preferably in the early morning, keeping the husks on, and are taken in this condition to the factory. They are husked and the silks removed and passed through machines with sets of knives which cut the grains evenly from the cob, care being observed not to cut the corn so closely as to cut off particles of the cob with the corn. In some cases the cobs are next passed through scrapers which remove the small tips adherent to the cob. After the corn is cut it is run through a cleaner which removes bits of cob, husk, and silk. It is then passed to a mixer and the proper amount of water, bearing sugar and salt in solution is thoroughly stirred through the mass. It is then run into the filler and cooker. Most of the operations are done by machinery, and the different processes follow each other in such rapid succession that from the time the ear goes into the husking machine until the corn is in the can, sealed, and ready for the retort may not be more than fifteen minutes.

Peas.—In the pea-canning industry the vines are cut with a mower or a special pea harvester, loaded onto racks and hauled to the vining machines. The viner is a machine consisting of an outer and an inner cylinder revolving in opposite directions, the inner one bearing paddles or beaters so arranged that as the vines pass through the machine the paddles break open the pods. As the peas are thrown out, they pass through perforations in the outer cylinder, while the vines are discharged at the opposite end. The peas are run through a fanning mill to blow out bits of stems, leaves, and pods after which they are washed to remove all dirt and also the mucous substance from the surface thus insuring a clearer liquor in the can. The peas are next passed through a sizer, which separates them into five sizes or grades. Some peas are packed ungraded and the proportion thus packed is increasing. The peas are next blanched to drive water into them so that all will be tender. The time of blanching varies from one-half to five or more minutes, large mature peas requiring more time for the blanching than young tender ones. The peas are then filled into cans by machines which deliver exact quantities together with the necessary brine after which they are processed.

Fruits.—The essentials in the canning of fruits do not differ from those for vegetables. Stone fruits may be canned either with or without the pits. In the case of such fruits as cherries, or other acid fruits, the tin is coated on the inside with a lacquer or enamel which protects the tin from erosion by the action of the acid juices. The time and temperature of processing fruits is usually less than that required for vegetables, for the reason that in the presence of the fruit acids the organisms are more easily destroyed than in foods in which acids are not present.

CONTROLLING FACTORS IN SUCCESSFUL CANNING

CLEANLINESS.—Too much emphasis could hardly be placed upon the importance of cleanliness throughout the whole preserving process, and especially in the preparation of the food for preserving. Vegetables that have come into contact with the soil are pretty certain to harbor many spores of bacteria, and if as many of these are removed as possible by a thorough preliminary cleansing, processing may be effected with greater ease and certainty. The necessity of cleanliness on the part of factory employees is needful only of mention, not only from the esthetic standpoint, but also from that of good health.

THE SOUNDNESS OF RAW MATERIAL.—The necessity of sound and wholesome raw material is fully as great as that of cleanliness in handling. Foods are never better than when they are fresh. It makes no difference how long nor by what method they may be cooked, the quality cannot be bettered, and if food is unsound when put into the containers for canning, it will never be wholesome for food; and this fact is equally true whether the unsoundness is the result of diseased conditions of meats, fruits, or other products, or whether it is due to ordinary decay.

WATER SUPPLY.—Another essential for the success of the canner is an ample supply of pure water. It is a well-known bacteriological fact that outbreaks of spoilage have occurred in canneries which could be traced to organisms getting into the goods from the water supply.

RECEPTACLES.—The commercial canner recognizes two essentials for suitable containers for his goods. First, they must be tight, both to prevent the escape of the contained material and the entrance of contaminating organisms. Second, they must be of a material which will withstand erosion or corrosion for a reasonable length of time, without giving up any notable quantity of foreign material to the food with which they may be in contact. Glass is most satisfactory from this consideration, but for reasons previously stated it is impracticable for use on a commercial scale. The difficulty from erosion in tin cans has been largely overcome by the use of enamelled cans as mentioned above.

DEGREE OF HEAT REQUIRED.* *Factors in Processing.*—The heating of food products after placing in the containers is termed *processing* by the commercial canner, and he appreciates fully that upon the care with which the processing is done depends the success of the entire pack.

The several factors which enter into the successful processing of foods, either in commercial or home canning may be enumerated as follows: (*a*) length of time, (*b*) number and resistance of spores present in the material, (*c*) size of container, (*d*) consistency of contents of the container, (*e*) initial temperature, (*f*) agitation of the container during processing.

Length of Time Required.—Fig. 152 gives the curves showing the time necessary at various temperatures to destroy the spores of three of the most resistant organisms found in canned foods when about fifty thousand spores per c.c. are present. It will be noted that a drop of ten degrees Centigrade necessitates about ten times as long for the destruction of the spores. For instance in organism No. 26 six minutes at 125°C. are necessary and about sixty-five minutes at 115°.

Number and Resistance of Spores.—Fig. 153 shows the influence of the number of spores on the processing time. It will be noted that organism "C" when about twenty spores were present required twelve minutes for their destruction; when about fifty thousand spores were present, sixty minutes were necessary. In other words, at a temperature of 115°C. nearly six times as long was found to be necessary

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* The author is indebted for charts and data to Dr. W. D. Bigelow, Chief Chemist of the National Canners Association.

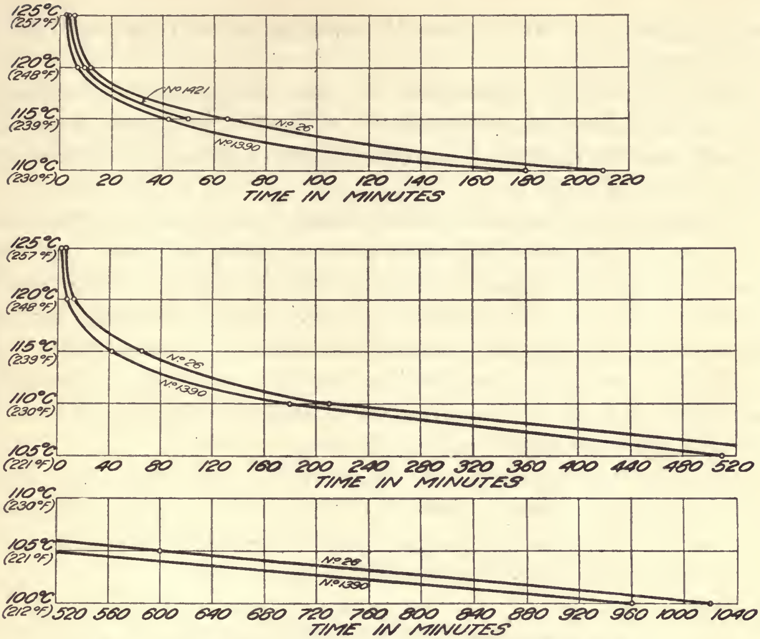

FIG. 152—Influence of temperature on sterilizing time.

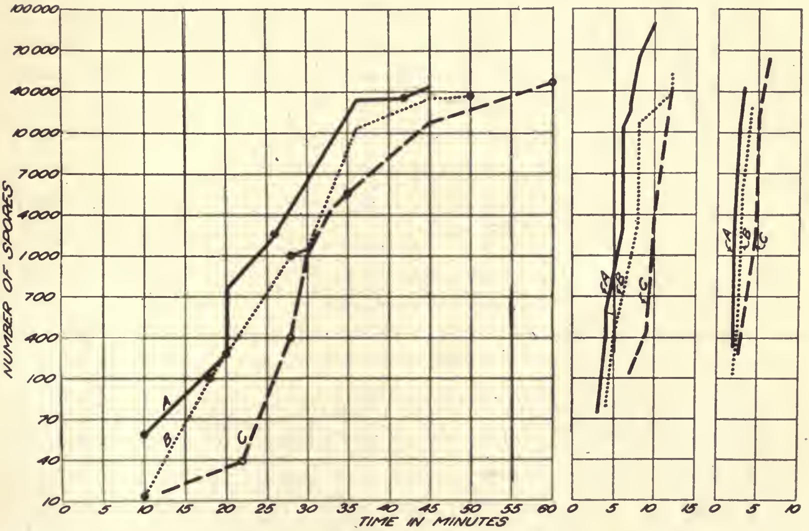

FIG. 153.—Influence of number of spores on sterilizing time.

in the presence of fifty thousand spores as in the presence of about twenty spores.

Size of Container.—Assuming the same food product, it naturally follows that a large can would require a longer time for processing than a small one, the rapidity of heat penetration varying according to the size of the can.

Consistency of Contents of the Container.—Consistency is expressed in the way it is determined when a can is examined after it has been packed, that is, in terms of drained solids. A can containing twenty-seven ounces of drained solids will require a longer processing time than the same size can containing only eighteen ounces of drained solids. Or, to apply the principle in another way, a product such as peas or carrots will require less time for heat penetration than corn or spinach in which the food is packed more closely in the can.

Initial Temperature.—Foods filled into the cans hot can be successfully processed in a shorter time than if packed cold. This is especially true in the case of such goods as corn in which the heat penetration is slower than with some other products such as peas.

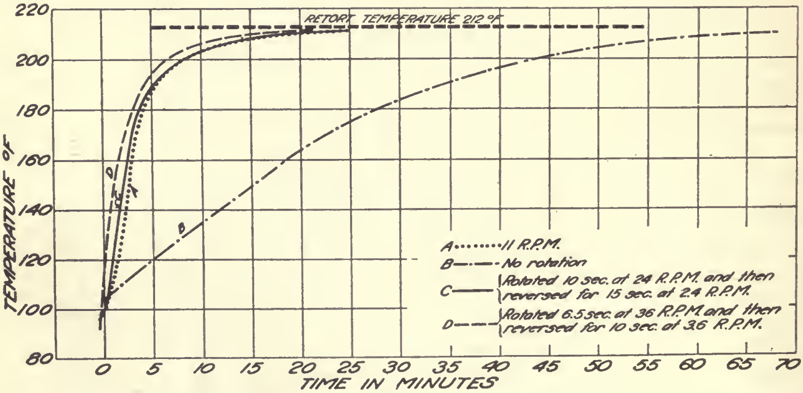

FIG. 154.—Tomatoes in no. 3 cans—Influence of speed of rotation on heat penetration.

Agitation of the Container during Processing.—This factor can best be explained by Fig. 154 which shows the influence on heat penetration of rotating a can of tomatoes while it is being processed. The can represented by curve "B" was processed in the ordinary steel retort without rotating. The can in curve "A" was processed continuously at eleven revolutions per minute. The cans in the curves marked "C" and "D" were processed according to the practice of one of the typical commercial cookers. It will be noticed that whereas the center of a #3 can of tomatoes required about twenty-eight minutes to reach the temperature of 180° without rotation, it reached the same temperature in three or four minutes with rotation. In general it may be said that a product consisting of solid particles surrounded by a relatively clear liquor heats to the center of the can almost as quickly as if the particles were not there, that is, almost as quickly as water in a still retort so that agitation is not of particular value in such products. A product of a viscous nature which interferes with convection currents, however, heats to the center of the can much more quickly if there is some form of agitation which supplements the convection currents. By rotation as mentioned above is meant the rotating of a can on its axis as done by some of the commercial cookers now widely used with tomatoes and some other products.

Home Canning of Foods

The successful canning of foods in the home depends upon the same principles as those employed in commercial canning, namely, cleanliness, soundness of raw material, and thorough processing. Aside from the universally used open kettle method of handling the foods, two other methods are now widely used: the cold-pack method, and the vacuum-seal method. In the cold-pack method, the products are cleaned, blanched by dipping in hot water, and immediately packed into the glass jars or other containers. To fruits hot syrup may be added; to vegetables and greens hot water and a little salt is added. Preservation of fruits and some vegetables may be effected by the intermittent or fractional method of heating on each of three successive days, the time of heating depending on the nature of the product and the size of the container as described above. The extra time, labor, and fuel required for this method makes it impractical where a large amount of canning is to be done. Furthermore, as Bigelow and Estey have shown, foods may carry organisms the spores of which are not destroyed by fractional processing, and these may cause spoilage of the canned product. The more common method of home canning is to process the food in one period by immersion of the containers in water which is then brought up to the boiling point; or by the use of steam pressure outfits of which the market affords several types. These operate on the same principle as the laboratory autoclav or the commercial canner's retort.

Spoilage of Canned Foods

Microbial changes occur when the goods have not been processed at a temperature sufficiently high to destroy all the organisms present in the uncooked food, or when a vacuum has not been established. In some instances, the organisms decompose the contents of the can with formation of gas, causing bulging of the ends of the cans sometimes to the point of bursting at the seams. Such cans are designated at the factory as "swells." In other instances, the bacteria cause an acid fermentation with consequent souring of the contents. The canner terms such cans "flat sours."

Detection of Spoiled Goods.—In cases of spoilage accompanied by gas production, detection of the spoiled cans is easy from the bulged appearance of the ends of the cans. On account of the exhaustion of air from the cans previous to processing, the ends of sound cans should be slightly concave. If the ends of the cans are convex, it indicates some abnormal condition of the contents and such cans should be rejected. In the case of sours, detection is not so easy. The can may appear normal, and there may be no change in the contents apparent to the eye on opening the can. Taste, however, reveals a more or less pronounced disagreeable acid flavor. Canned meats, fish, or crustaceans are likewise liable to spoilage if the processing has been imperfectly carried out. In these goods the change is generally accompanied by gas production, hence detection is easy because of the swelled appearance of the cans.

If gas production is present, or there is an odor resembling rancid cheese, or if the contents appear mushy or disintegrated, in no case should the contents of the can be tasted to see if it is spoiled, as these conditions indicate spoilage by *B. botulinus*, and the toxin of this organism may prove fatal in the smallest traces.

Disposal of Factory Refuse

The disposal of factory refuse has at times been a serious problem for the commercial canner. Of late years methods have been devised for utilizing much of the material that formerly was allowed to accumulate about the factory in fermenting heaps to the extent of sometimes becoming a nuisance to the neighborhood.

At pea canneries several methods of utilizing the vines are in use. They may be converted into silage, either by putting into silos or stacking in large stacks. In some sections the vines are cured for hay. They are also valuable as a fertilizer.

Corn husks and cobs are also used for silage. Experiments were made by the United States Department of Agriculture in regard to the feasibility of using the refuse from the canning of corn for the production of alcohol. It was found that, on account of the expensive machinery and apparatus required in the manufacture, a small factory could not profitably utilize the corn waste for alcohol. It was shown that where several factories were located within a short radius of each other, by shipping their waste to a central plant, it might be used up to advantage.

Apple cores, "chops" and peelings are usually either used for vinegar making, or are made up into apple jelly. From one factory visited by the writer, the apple cores and peelings were dried, baled, and shipped to Europe, "to be made up into champagne."

Peach pits are sometimes sold to nurserymen for seed. Sometimes the pits are cracked and the meats used for almond meats and also oil. In many factories, no use is made of the peach stones.

In the classes of foods in which the waste is not large, the refuse is hauled away to a dumping ground near the factory, or is taken away by farmers for its manurial value.

CHAPTER III*

THE PRESERVATION OF FOOD BY COLD

INTRODUCTION

In recent times cold storage has become of very great importance in the preservation of perishable food stuffs, and foods preserved by cold usually command a higher market price than those preserved by other methods. This is probably due primarily to the fact that the general appearance of refrigerated food resembles that of the perfectly fresh article, in many instances very closely. Moreover, in many instances cold storage, for a reasonable length of time, preserves not only the appearance and the nutritive value, but also the chemical composition and even the delicate flavors of the original articles, so important in determining market value. The great economic importance of this industry is at once apparent, for it aims to preserve unchanged the over-abundance of one locality for transportation to another and the over-production of one season of the year for subsequent use.

THE EFFECTS OF REFRIGERATION UPON FOODS IN GENERAL

The decomposition of foods depends upon the activity of their own intrinsic enzymes to some extent, but more especially upon the activity of foreign microörganisms—bacteria, yeasts and molds. Cold acts as a preservative, not by destroying these microbes, but by retarding or inhibiting their activity. In general, cold not only retards the growth of the microörganisms but delays their death also, tending to preserve them as well as the food unchanged.

In discussing the refrigeration of foods we may consider three periods of treatment, (1) the removal of the heat or chilling of the food, (2) the prolonged storage at low temperature, (3) the subsequent warming of the food before sale or consumption.

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* Prepared by W. J. MacNeal.

Changes during Chilling.—The period of cooling is a relatively short one, varying from a few hours to a few days in length. The chief physical change is the intentional removal of heat by conduction and convection, but there is usually also some loss of water by evaporation. If cooled to a sufficient degree the water content of the food may crystallize, altering to a considerable extent the physical structure of the food substance (frozen food). Most foods are either actually living when chilling begins, or they are only recently dead and various chemical changes due to intrinsic enzymes continue at a diminishing rate as the heat is removed. Decomposition changes, due to microbes, may also be in progress and continue during the process of chilling. At this time the microbes living in the cold-storage chamber gain access to the newly arrived food and others are added in the process of handling. The extent to which these will grow and multiply depends upon their ability to flourish under the storage conditions. In general the bacteria which flourish at ordinary temperatures, producing the familiar decomposition of the particular food, are greatly retarded in their activities and other kinds of microbes outstrip them under the new conditions. The changes taking place during chilling are of great importance in some special instances, and often a very definite procedure must be followed to obtain a satisfactory result.

Changes during Storage.—This is often a very long period so that causes acting very slowly may ultimately produce marked alterations. There is ordinarily some loss of water by evaporation, as well as the evaporation or diffusion of other volatile constituents, some of them at times important factors in the flavor of the food. Other volatile substances may be absorbed from the air of the storage room introducing undesirable odor or flavor. The chemical changes of the chilling period continue at a greatly diminished rate, or may be entirely inhibited if the food is frozen. The behavior of the microbic content of the food is the most important factor to be considered during this period. Besides those already present, various other microörganisms, bacteria, yeasts or molds, may gain access to the food from time to time, either from the circulating air or by contact with other things. The fate of the implanted microbes will depend upon their nature and adaptation to the conditions existing in the stored food. Many of them perish, but many also survive the entire period of storage, and some may actively multiply. There can no longer be any doubt that some bacteria can grow at the temperature of zero, and many kinds multiply at a fraction of a degree above that point. In order definitely to inhibit microbic activity the food must be frozen. When it is not frozen, bacteria continue to multiply slowly at the lowest temperature of storage, and small variations in the temperature and in the humidity of the atmosphere serve to accelerate their activity. Such variations also accelerate diffusion currents in the food substance and so tend to distribute the microörganisms and their products. The extent of the resulting chemical changes in the food will depend upon these factors and upon the nature of the food, the temperature and the length of the period of storage.

CHANGES AFTER STORAGE.—This is a relatively short period, but in many instances a very important one as regards change in the food. If warmed too rapidly, vigorous currents may be set up in the food mass by the great difference in temperature between the outer portion and the interior, serving to distribute microörganisms and their products. In the case of frozen foods rapid warming fails to restore the original physical structure. Dry cold foods are likely to condense moisture from the warmer atmosphere unless it is particularly dry, and this condensed water becomes another cause of diffusion currents. In frozen foods the water, in melting, may fail to reënter the food structure, and exude and drip away, carrying a portion of the soluble constituents with it. At this time, still more microbes are likely to be added to the food, and, together with those already present, they multiply with increasing rapidity as the temperature rises. As they may already be pretty well distributed throughout the mass of the food, the resulting chemical decomposition is the more rapid. It is well recognized that, in keeping qualities, foods removed from cold storage are much inferior to the corresponding fresh foods.

REFRIGERATION OF CERTAIN FOODS

MEAT, FISH AND POULTRY.—Meat, in this sense the flesh of mammals, is preserved by cold in two ways, by storage above the freezing-point (chilled meat) and by storage at −10° to −4° (frozen meat). Fish and poultry are usually frozen for storage, often in the undrawn condition.

Mammals killed for chilled or for frozen meat are slaughtered and carefully dressed. For chilled meat the temperature is reduced by storage in a cold air chamber to about +2° in forty-eight hours, and the meat is stored at a temperature between +1° and +2°. Under these conditions the enzymes of the dead flesh continue to act and bacterial decomposition proceeds slowly, bringing about a process of ripening which, up to a certain point, improves the market value of the flesh by making it more tender and giving to it a more desirable flavor. The extent to which the slow bacterial decomposition may proceed before the flavor becomes disagreeable varies with different tastes, but in general the beginning of proteolytic change, which follows after the almost complete fermentation of the muscle sugar, may be said to mark the desirable limit. This point is reached in from a week to three months, depending upon the condition of the animal, skill and care in slaughter and dressing, especially the extent of bacterial contamination at this time, and the accurate control of the storage conditions. Fresh killed beef is generally regarded as quite inferior to it. In the production of frozen meat the carcasses are rapidly chilled in an air chamber at −20°, where the meat remains until frozen solid. It is then kept at a temperature below −4°. Freezing produces a marked change in the finer physical structure of the meat, as the water crystallizes, leaving the protein material, with which it was formerly intimately mixed, in a shrunken and shriveled state between the crystals. Enzymic and bacterial activities are practically if not absolutely suspended under these conditions, and, save for slight surface evaporation, such meat remains unchanged for long periods. The subsequent thawing presents certain difficulties and requires particular care. If warmed very slowly the melting water crystals are imbibed by the protein material and the original structure of the flesh almost completely restored. The warmer air must be dry and must be kept in motion to avoid condensation of moisture on the exterior of the thawing meat. Bacterial activity is likely to gain considerable headway during this process and the penetration of the microbes into the flesh is favored by the diffusion currents. The more prolonged the warming process, the greater the opportunity for bacterial decomposition. Ordinarily, to avoid this, the thawing is carried out rapidly and the finer structure of the meat is not restored. It is softer, darker and more moist than fresh or chilled meat, and usually sells at a lower market price.

It is preferable that frozen meat should always be marketed as such and should come into the hands of the cook while still frozen hard. As soon as portions become soft they should be cut off and cooked. The extensive use of frozen beef during the war has proven so generally satisfactory that previous prejudice against this kind of meat has been largely forgotten. When once thawed it should be used promptly.

Fish and poultry are usually frozen for storage. As these foods are especially subject to rapid objectionable decomposition changes they are rapidly chilled in ice water or by packing in ice immediately after death, and are frozen as quickly as possible. During storage in the frozen condition microbic activity is suspended, but in the subsequent thawing the same physical and biological changes occur as in frozen meat. When fish and poultry are stored in the undrawn condition there is an abundant supply of bacteria at hand in the intestinal contents ready to multiply energetically during the chilling and thawing stages. It would appear desirable that the poultry should be killed and dressed with great care previous to freezing and that the period of chilling should be shortened as much as possible. Practically, however, it has been found that the dressing of poultry, as ordinarily done, previous to storage, leads to such an extensive soiling of the edible flesh of the birds that their condition at the end of the storage period is often less satisfactory than that of undrawn frozen poultry, not only in gross appearance but also in respect to microbic content and chemical composition. Most frozen poultry is, therefore, stored in the undrawn condition.

The tendency of such food to undergo decomposition after thawing should be clearly recognized and prompt cooking at once after softening should be insisted upon. Its sale as fresh or as chilled food is a fraud upon the purchaser. In fact many individuals seem to be peculiarly liable to suffer digestive disturbances after eating frozen poultry and such persons should avoid its use.

The nature and source of the bacteria which produce poisonous changes in poultry are not definitely known, but there is some evidence indicating that they belong to the para-colon group and that they are derived from the intestinal contents of the fowls. Smith and TenBroeck* have studied a typhoid-like bacillus found in the intestinal contents of fowls. This organism produces a poison which is only partially destroyed by boiling for 15 minutes. It may be suggested that organisms of this type existing in frozen poultry might well account for poisonous effects produced by the flesh after roasting or boiling.

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* Smith and TenBroeck, Journal of Medical Research, Jan., 1915, Vol. 31, No. 3, pp. 523–546.

Eggs.—The cold storage of eggs is an industry which has attained large proportions in recent years. A very constant storage temperature between +0.5° and +1° is essential for the best results. The humidity of the atmosphere is also of very great importance, as a dry air causes extensive evaporation from the egg and a too moist air favors the development of microörganisms on the exterior of the shell and the absorption of their products and even their penetration into the egg. A constant humidity of 70 per cent saturation has been found to be the best. Storage at this temperature and humidity greatly retards the growth of microörganisms and definitely inhibits the ordinary putrefaction of eggs. The activity of the intrinsic enzymes of the egg are not necessarily inhibited by this temperature, nor is the growth of all microörganisms prevented. Unquestionably there is a marked difference between the ordinary cold-storage egg and the strictly fresh egg, but to what extent this deterioration may be due to errors in storage, such as inaccurate control of temperature and humidity, use of odoriferous crates for packing, decomposition changes previous to storage, too rapid chilling of the eggs, or too rapid warming of them after removal from storage, and to what extent it is inherent in the most perfect cold-storage procedure, is still somewhat uncertain. Doubtless a certain amount of deterioration, especially the loss of the peculiar flavor of the fresh egg, is unavoidable in any method of prolonged storage. The discrimination in price in favor of new-laid eggs in the market is an indication of difference in actual value, and the sale of cold-storage eggs for new-laid or strictly fresh eggs is generally recognized as a fraud by the purchaser. The cold-storage egg is nevertheless a very valuable food and the economic importance of saving the overabundant supply produced during the spring for use during the colder season of the year makes this industry a great benefaction to the public. Suitable regulation may be expected to remove its objectionable features.

Milk and Butter.—Milk as ordinarily sold at retail is not subject to sufficient seasonal change in market price to make its prolonged storage advisable. But milk is so rapidly changed by bacterial activity at ordinary temperatures that efficient dairy methods necessarily include prompt cooling of the milk after it is drawn from the animal and the maintenance of a low temperature until it is delivered to the consumer. At the low temperature bacteria slowly multiply, unless the milk is actually frozen, but at a temperature slightly above the freezing-point very clean milk may be kept in perfect condition for a week, and it may be kept sweet for several weeks. Refrigeration of milk cannot compensate for unhealthy animals producing it, nor for careless and uncleanly methods of handling. The cold does not destroy the microbes in the milk but only retards their multiplication and chemical activity. In practice, especially in the transportation of milk into large cities, it is frequently most economical to freeze the milk and trust to insulation and the latent cold in the ice to maintain a low temperature during transportation. Such milk should arrive at its destination in a partly frozen condition.

The cold storage of butter is essential even when it is kept for only short periods, and the seasonal variation in price is sufficient to warrant its storage from summer to winter. The keeping qualities of butter depend upon many factors,* and the most efficient cold storage cannot compensate for previous deficiencies. In refrigerated butter there is a gradual diminution in the number of living bacteria, with possibly a multiplication of a few particular kinds. There is a slow increase in acidity. In frozen butter the bacterial content and the chemical composition remain practically unchanged.

Fruits and Vegetables.—These foods are for the most part adapted to preservation for short periods at ordinary temperatures, and cold storage at a temperature slightly above zero is very effective in diminishing the rate of change in them. The humidity of the storage chamber should be kept constant at about 60 per cent saturation in order to diminish evaporation as far as possible without favoring the development of molds. These foods generally remain alive during storage and the changes due to intrinsic enzymes are often important. Some fruits need to undergo further ripening in storage before they are ready for consumption and this change may be accelerated or delayed by changing the temperature of the storage chamber. The development of bacteria and molds with consequent rotting is best delayed by maintaining dry clean fruits and vegetables in an atmosphere of very constant humidity and very constant temperature slightly above the freezing-point.

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* See chapter on the microbiology of butter.

LEGAL CONTROL OF THE COLD-STORAGE INDUSTRY

There has been a rather widespread prejudice against cold-storage food products, and in some respects this is not without justification. Cold storage preserves so well the external appearance of fresh foods that deception in the sale of them to the consumer has been too frequently practised. This is extremely unfortunate for all parties concerned in such transactions. The proper branding of all cold-storage foods, clearly indicating their character and the length of time held in storage, would ultimately benefit the producer, the consumer and also the cold-storage industry. Where cold storage is efficient such a practice would proclaim its efficiency. Where it is inefficient the cold-storage industry can ill afford to allow the consumer to be deceived concerning the food he is purchasing. The strict enforcement of laws compelling the proper labeling of such foods and prohibiting their sale except when branded as such would quickly remove unjust prejudice against cold storage, and would place this industry upon a secure foundation, greatly increasing the possibilities of its service to the food producers and consumers, and at the same time promoting the legitimate interests of the cold-storage industry.

CHAPTER IV*

THE PRESERVATION OF FOOD BY CHEMICALS

The addition of preservative substances to foods is a very ancient practice, and as no extensive equipment is required it is one of the cheapest ways of preserving food, especially on a small scale. The resulting alteration of the food in appearance and composition is greater than when it is preserved by cold storage, for the preservative substance added becomes a more or less permanent constituent of the food, but the changes are not necessarily undesirable. The addition of chemical preservatives is often practised in conjunction with desiccation or cold, or sometimes even in canned or bottled foods sterilized by heat. All the substances employed as preservatives owe whatever efficiency they may possess to their ability to restrict the activity of microörganisms, that is, their antiseptic properties.

The Effects of Preservatives upon Foods in General

In only a few instances are chemical preservatives added to foods to be sold as fresh foods, and these practices are generally regarded with disfavor. Their most important use is in the prepared foods, the preservative being incorporated with the food during the process of preparation for storage.

The Process of Curing.—The procedures employed necessarily vary with different foods. Physical alterations in the food, such as changes in form, texture and water content are usually involved, as well as the solution of the preservative in the juices of the food. Chemical changes due to the intrinsic enzymes of the food, to the various accessory procedures such as drying, cooking or soaking in pickling solution may produce marked alteration. In some cases the preservative reacts chemically with some constituent of the food. During the curing process microbic activity may be more or less prominent at various times, playing its part in the chemical changes.

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* Prepared by W. J. MacNeal.

Bacteria, yeasts and molds are likely to be introduced into the food by the various manipulations and some of these may find conditions favorable for their proliferation. In some instances the activity of certain kinds of microbes appears to be essential to the proper curing and subsequent adequate preservation of the food; the preservative, the constituents of the food and the microörganisms mutually reacting to bring about the desired result. It is worth noting that the added chemical preservative is never sufficiently potent to destroy with certainty pathogenic microbes which may be present in the food.

THE PERIOD OF STORAGE.—Unless the food has been sterilized and stored in sealed containers, slow changes in water content, in physical appearance and in chemical composition usually take place during storage. The added preservative may continue to react with the food substance and its decomposition products. During this period there is relatively little intimate manipulation of the food and therefore little opportunity for the penetration of new microbes. Some of those already present may continue their activities at a diminished rate, producing slow chemical changes often of a desirable nature rather than otherwise. Accessory conditions, such as desiccation, cold storage, or sterilization and sealing, may greatly retard or check altogether microbic activity.

THE AFTER-STORAGE CHANGES.—The immediate preparation of preserved food for consumption is frequently important. The preservative may be largely removed mechanically, or extracted with water. During cooking peculiar chemical reactions may occur, and cooking is also important in the destruction of microörganisms remaining alive in the food up to that time.

THE CHEMICAL PRESERVATION OF CERTAIN FOODS

MEATS AND FISH.—The preservation of meat and of fish by salting depends largely upon the increase of osmotic tension in the food, a physical change sufficient to prevent or greatly delay the growth of microörganisms. Sodium chloride ($NaCl$) probably owes its preservative value solely to this physical effect. In practice its action is often supplemented by the addition of a small amount of saltpeter (KNO_3), and sometimes also cane sugar ($C_{12}H_{22}O_{11}$). The fluids of the flesh are in part removed by this treatment, carrying away a part of the soluble constituents. The fluids which remain contain the added preservative substance in solution, and the whole mass of food substance is permeated by them. Potassium nitrate (saltpeter) reacts with the flesh, being reduced in part to nitrite. This enters into a combination with the coloring matter of meat, which upon cooking produces the characteristic red color of meat cured with saltpeter.

The various manipulations during the process of pickling or dry curing serve to introduce numerous microörganisms. Many of these may flourish in the pickling fluids, but in a sufficient concentration of salt and at a sufficiently low temperature, decomposition ordinarily does not progress so as to become objectionable, and proteolytic decomposition (putrefaction) is effectually prevented. This protection of the protein depends to some extent upon the acidity of the medium, which in turn is due largely to the bacterial decomposition of the muscle sugar. The powerful putrefactive bacteria (*B. œdematis* group) flourish only in an alkaline medium. On the other hand, too high a degree of acidity becomes in itself objectionable on account of the sour or rancid taste, and it is, therefore, important that the acid-producing bacteria should be held in check somewhat. In practice, saltpeter has proved of value for this particular purpose, and its action apparently depends upon the antiseptic effect of minute quantities of nitric acid (HNO_3) and nitrous acid (HNO_2) set free from the salt by the excess of organic acids produced by the bacteria. The curing of meats by pickling solutions is often supplemented by desiccation and impregnation with the antiseptic substances of wood smoke

The dry-salting of codfish is an example of preservation by increasing the osmotic tension. The fish is cleaned and beheaded, split longitudinally, and the vertebral column removed. It is then carefully washed, and all visible blood is removed. The pieces are next covered with dry salt and packed in open casks. The salt rapidly extracts water from the flesh and a strong brine results. After a few days the casks are emptied out, and the pieces of fish, now smaller because of the loss of water, are again thoroughly washed and again packed in dry salt so that the adjacent pieces of fish are completely separated by an intervening layer of solid salt. The contents of the cask are subjected to high pressure to remove air, and the cask is finally closed.

The curing of ham is an example of preservation by increased osmotic tension combined with the addition of chemical preservatives. After slaughter and chilling, the hams are injected with a solution containing 25 per cent common salt, 15 per cent granulated sugar, and 12 per cent saltpeter, and are then stored at a low temperature, preferably between 0° and +4°, in a brine containing about 20 per cent common salt, 5 per cent sugar, and 1 per cent saltpeter. The brine is renewed once or twice at intervals of a week or ten days. After about a month the hams are washed in warm water, dried and hung in wood smoke for several days. They are then stored in a cool place. The proportions of the various constituents of the pickling solutions are subject to rather wide variation, and in general, it may be said that the higher the temperature of the storage room, the more concentrated must be the pickling solutions to insure satisfactory preservation.

DAIRY PRODUCTS.—Butter is usually salted with sodium chloride to impart the desired taste, and this salt also acts to some extent as a preservative by increasing the osmotic tension of the moisture remaining in the butter. Antiseptics such as boric acid, saltpeter, salicylic acid and formaldehyde have been employed in the preservation of butter, the first-mentioned appearing to be the most satisfactory. One half of 1 per cent of boric acid incorporated with high-grade butter previous to storage greatly delays rancid change.

Fresh milk and cream are also sometimes treated with antiseptics such as formaldehyde, but the use of any chemical preservative whatever in these dairy products is unnecessary and generally disapproved.

PREPARED VEGETABLE AND FRUIT FOODS.—These foods are sometimes preserved by vinegar, sugar or alcohol, the presence of which is of course very evident to the consumer. Other substances less readily detected, such as sulphurous acid and sulphites, boric acid, salicylic acid, benzoic acid and sodium benzoate, and formaldehyde, are also employed in foods which must be kept some time after exposure to the air. These substances are incorporated with the food before it is packed, and serve to inhibit the activity of microörganisms which gain access to it.

THE NUTRITIVE VALUE OF PRESERVED FOODS

The nutritive value of a food depends upon the amount of utilizable food principles it contains. The food-principle content can be readily measured by chemical analysis, and in general there is no important difference between a preserved food and the corresponding fresh food in this respect. The utilization of the food principles, however, depends upon a number of factors and may be greatly influenced by individual peculiarities of the consumer. One important factor in the utilization of a food, and probably the most important factor in determining its market value, is palatability. In general, preserved foods are pleasant to the taste when eaten at intervals, but upon long continued daily ingestion, the appetite for them fails and they may even become distasteful. It would, therefore, appear to be erroneous to regard preserved foods as in every respect as valuable from the standpoint of nutrition as the corresponding fresh foods. The difference is not dependent upon a change in the food-principle content, but must be sought rather in slightly altered composition of the food and the specific effects of newly formed substances, and especially in the possible effects of the continued ingestion of the contained chemical preservatives upon the consumer.

THE EFFECTS OF FOOD PRESERVATIVES

The essential characters of a food preservative include antiseptic action to prevent decomposition of the food, and absence of evident poisonous or deleterious influence upon the consumer. It follows therefore that the effects of food preservatives upon the consumer, if they exist at all, are at any rate not easily recognized, and on account of the economic importance of the questions here involved, this field of scientific research has been energetically cultivated by investigators with different viewpoints, and the results of investigation have been discussed with some heat. The passage of the U. S. Food and Drugs Act was followed by considerable discussion of these questions. Gradually the practical administration of the law has become more settled and the use of many food preservatives is still permitted.

SUBSTANCES WHICH PRESERVE BY THEIR PHYSICAL ACTION.—The preservative effects of sodium chloride seem to depend upon the high osmotic tension of strong salt solution, and the same may be said of cane sugar. When diluted so as to be eaten with relish, these substances are themselves properly classed as foods, without deleterious effects upon ordinary individuals.

SUBSTANCES WHICH PRESERVE BY THEIR CHEMICAL ACTION.—These preservatives inhibit the activity of microörganisms in a different way, not by withdrawing water from the microbic cell, but by entering into chemical combination with the living substance in such a way as to hinder its activity, or by entering into chemical reactions with the food to produce new substances capable of attacking the microbic protoplasm in this way. The ideal chemical food preservative would be one which, without altering the food substance, would exhibit this poisonous property toward living protoplasm until the food was ready for consumption, and then would suddenly and permanently lose this property. None of the ordinary food preservatives approaches this ideal very closely.

Inorganic Food Preservatives.—Boric acid and borax are weak antiseptics, practically a saturated solution of boric acid being necessary to inhibit ordinary bacterial growth. When employed as a dry powder on the surface of meats, boric acid prevents the growth of mold, and most of it is removed from the food before consumption. When incorporated with butter it is eaten, and 0.5 to 1.0 g. may be taken daily in this way alone. The effect of such amounts of boric acid upon the consumer is still a disputed question. Wiley,* after an extensive investigation, concluded that small doses of either boric acid or borax continuously administered for long periods create disturbances of health.

Nitric acid and nitrous acid and their salts are food preservatives of some theoretical interest because it is well known that some bacteria readily decompose fairly strong solutions of nitrates, and also oxidize or reduce nitrites. Apparently, however, this is true only in neutral or alkaline solutions, and in the presence of free acid the activity of these microbes is quickly inhibited. The preservative effect of nitrates and nitrites is best ascribed to the liberation of minute quantities of free nitric and nitrous acids from these salts, and these substances are without value as preservatives in foods which are alkaline in reaction. The effect of the ingestion of nitrate or foods preserved with nitrate upon the consumer has been investigated by Wiley, who concluded that the deleterious effects are slight and less clearly detected than in the case of the other preservatives. Small quantities of saltpeter appreciably delay the digestive action of a mixture of pepsin and hydrochloric acid (artificial gastric juice) upon coagulated egg white in a test tube.† Minute but variable amounts of nitrites occur in foods preserved with nitrates, but whether these amounts are sufficient to produce the specific nitrite effect upon the blood circulation of the consumer has not yet been definitely ascertained.

Sulphurous acid and the sulphites are rather extensively used in chopped meat (Hamburg steak) and in cider and wines. The addition of sulphite to chopped meat serves a three-fold purpose, retarding bacterial decomposition, producing a red color on the exposed surface, and removing odors of decomposition. It thus not only delays decomposition, but also to a certain extent masks the decomposition which has already occurred. The ingestion of moderate quantities of sulphites in food has at times been followed by acute gastric derangement in man, and prolonged feeding of meat containing sulphites has been followed by inflammatory changes in the kidneys of experimental animals.

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* U. S. Dept. Agr., Bureau of Chemistry, Bull. No. 84, Part I.

† MacNeal and Kerr: Unpublished work.

Fluorides have been used to a slight extent in beverages, but acute gastric derangement and depression of the heart are caused by rather small quantities, and probably on this account the salts of hydrofluoric acid have not come into very general use as food preservatives.

Organic Food Preservatives.—Formic acid ($H \cdot COOH$) and acetic acid ($CH_3 \cdot COOH$) are produced by microbic activity and their preservative action appears to depend more upon the degree of acidity than upon the character of the acid radical. Both these acids appear to be utilized as food in the body of the consumer.

Benzoic acid and benzoates are rather extensively employed in prepared vegetable food products, such as jams and catsups. The antiseptic effect seems to be due wholly to free benzoic acid, even where it is added in the form of the salt, but the action is not due merely to the acidity (*i.e.*, the hydrogen ion). Benzoic acid is not utilized as a food in the body, but is excreted by the kidneys in the form of hippuric acid. It has been said to produce irritant effects upon the stomach and the kidneys, and to arrest the action of digestive enzymes in dilute solutions, but the Referee Board* of the United States Department of Agriculture, after extensive investigations, concluded that small doses of sodium benzoate mixed with food are not injurious to health, and do not impair the quality or nutritive value of the food.

Salicylic acid and the salicylates have been used for much the same purposes as benzoic acid, and there does not appear to be much difference between the two acids, either in their efficiency as preservatives or in their possible deleterious effects upon the consumer. Salicylic acid is more expensive. After extensive investigation Wiley† has concluded that the addition of salicylic acid and salicylates to foods is reprehensible in every respect, this conclusion corresponding to the results of similar work by the same investigator‡ upon benzoic acid.

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* U. S. Dept. Agr. Report No. 88, May, 1909.

† U. S. Dept. Agr., Bureau of Chemistry, Bull. No. 84, Part II, 1906.

‡ U. S. Dept. Agr., Bureau of Chemistry, Bull. No. 84, Part IV, 1908.

Formaldehyde is very efficient as an antiseptic, delaying microbial decomposition when added to foods in very small quantity. Its use for this purpose is generally condemned, partly because of its hardening or "fixing" effect upon the protein constituents of the food, tending to make them more indigestible. Its use in milk and milk products, though still practised to some extent, has been prohibited by law in some states.

Alcohol ($CH_3 \cdot CH_2OH$), in sufficient concentration, is an excellent preservative, but its presence in foods is readily detected, and it gives rise to characteristic effects upon the consumer. Furthermore, such foods are subject to special taxation as alcoholic products. Its use as a food preservative is therefore limited.

Wood smoke has been employed for centuries in the curing of meats. Its antiseptic properties probably depend for the most part upon creosote and pyroligneous acid, constituents of wood smoke which are antiseptic and also undoubtedly poisonous in sufficient doses. Smoking is a time-honored custom, however, and the amount of these substances consumed with the smoked meat is doubtless exceedingly minute.

SUBSTANCES ADDED TO FOODS TO IMPROVE THE APPARENT QUALITY. Several chemical substances are employed in various foods to improve the appearance, or to simulate the taste of a higher-grade product. In some cases the presence of these agents is known to the consumer, and desired by him; in other instances they are employed to deceive the purchaser. Butter coloring is quite generally used to produce the color of June butter the year around; nitrates bring about a pleasing red color in cooked pickled meats; copper sulphate is used to give a more brilliant green color to prepared vegetable foods; sulphites restore the red color of freshly cut meat to meat far from fresh; saccharine devoid of food value gives a taste resembling sugar to a variety of preparations at a great saving in cost to the manufacturer; carbonates of the alkalies or alkaline earths, added to milk, neutralize the acids resulting from bacterial decomposition and so keep the milk sweet; inorganic acids added to weak vinegar increase its acidity. Some of these practices are so universal and so well known that they are no longer criticized; others, such as the use of chalk in milk, are generally disapproved.

Legal Control of the Preservation of Foods by Chemicals

The desirability of legal regulation of the use of chemical food preservatives is now generally recognized, but there is still considerable diversity of opinion concerning what this regulation should be. Few, indeed, maintain that a substance exerting antiseptic action upon microörganisms outside the body is wholly without influence, after ingestion, upon the enzymes and bacteria of the normal digestive tract, even if we disregard the possible effects of the substance after absorption. It seems necessary to grant the existence of some effect, even though it be so slight as to have escaped detection. Over against the possible injury to the consumer must be placed the economic saving through the use of the preservative, often involving a considerable amount of money. In the absence of accurate and trustworthy knowledge concerning the actual influence of preservatives in the human body it would seem wise to prohibit all deception in regard to their presence. The principle advocated by Pasteur (1891) would still seem to be best, that is, to allow the use of preservatives, which are not known to be dangerous, upon the condition that their presence and the exact amounts be definitely and clearly stated on an appropriate label for the benefit of the purchaser and the ultimate consumer. Such regulation would not only protect the consumer against deception and fraud but would go far toward removing prejudice against preservatives, for even now there is little or no objection to those preservative substances of which the presence and the amount can be detected and roughly measured by the senses, such as salt, sugar, spices, vinegar and wood smoke.

CHAPTER V

MICROBIOLOGY OF FERMENTED FOODS

COMPRESSED YEAST*

It is stated that bread over four thousand years old from the tombs of ancient Egypt has been found to contain dead yeast cells, indicating the antiquity of the use of yeast in bread making. The leavened bread of the ancients doubtless contained yeast in combination with other microörganisms. "Potato yeast starters" for bread making were a later development but antedate the manufacture of compressed yeast as we now know this industry. With the development of brewing it was found that the yeast left in the fermentation vats was suitable for bread making. In England a great deal of brewers' yeast is still used for this purpose; housewives, rather than commercial bakers, are the principal users of this yeast. Experience has shown that brewers' yeast is weaker than distillers' yeast and, therefore, less suitable for breadmaking; brewers' yeast also is often very bitter from the presence of hop resins. Because of these facts commercial bakers came to prefer distillers' yeast, which at first was a by-product of the alcohol or potato spirits factory. This by-product yeast varied greatly in strength and general quality and a demand arose for a yeast of more uniform character. As a result of this demand suitable pure strains of yeast were selected and propagated for breadmaking—the alcohol in this case itself becoming a by-product and yeast the primary product. The compressed yeast and yeast cake industry has now reached great proportions and represents a very striking example of the successful application of science to industry.

Although the manufacturing processes are carefully controlled analytically and bacteriologically and although a great deal of valuable research work has been done in recent years, little of the information thus obtained has been made available to microbiologists. However, the Wahl-Henius Baking Institute and others are doing much to disseminate and to increase the existing knowledge on the subject.

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* Prepared by W. V. Cruess.

The manufacture of compressed yeast begins with the preparation of the malt. Two cereals, barley and rye, are commonly used in most factories—some use barley only. The barley is first "steeped" in cold water as for brewing (page 623). It is then placed in large slowly revolving drums maintained by a current of moist cool air at 10° to 25°. The air is cooled by being drawn through water sprays and wet coke. The usual temperature is about 15° to 18°. The barley is allowed to germinate until the *acrospire* (embryo blade) has reached the length of the kernel. Rootlets several times the length of the kernel form and cause the sprouted barley to mat together.

The object of malting is to cause the barley through the process of germination to form large amounts of diastase and of proteases. The formation of the latter enzymes is as important as that of diastase because the yield and vigor of the yeast depend upon the amount of assimilable nitrogen compounds present in the nutrient liquid. These are derived largely from the complex insoluble proteins of the grain by the hydrolyzing action of protein-splitting enzymes. Too short a period of too high a temperature of malting results in low yields of the enzymes; too long a period results in loss of enzymes formed during germination and loss of starch. Under normal conditions of temperature and humidity about five to seven days' malting is used for barley. A typical record of temperature and rate of rotation of a drum during malting is given by Wahl-Henius as follows:

Day	Temperature	Period of Rotation
1st	13°	Once in 2 hours.
2d	16°	Once in 2 hours.
3d	18°	Once in 2 hours.
4th	21°	Once in 1½ hours.
5th	24°	Once in 40 minutes.

Rye is said to be used to furnish protein and mineral salts which increase the yield and vigor of the yeast. It is malted on cement floors in layers about eight inches thick. Much less of the rye is used than of the barley. The sprouted barley and rye grains are crushed between rough steel rolls which revolve at different speeds, the difference in speed resulting in a grinding action upon the grains. The malt is not dried before grinding; in this respect the practice is different from that followed in brewing. Drying results in loss of enzyme-content and increases the cost of operation without in any way improving the quality of the yeast.

The crushed malt is mixed with water in a large wooden tank known as the "Mash tun" and which is equipped with revolving stirring arms and steam coils. The coils are usually controlled by a thermostat in order that an exact temperature may be maintained in the mash tun during acidification of the mash.

In the mash tun the starch is converted into maltose; a high concentration of lactic acid is formed and the insoluble proteins of the grains are converted to a considerable degree into peptones and to a certain extent into amino acids. The formation of acid and hydrolysis of the proteins are obtained largely by maintaining a temperature of 50° to 52°. A vigorous culture of a lactic acid forming organism is added to the mash as a starter. *B. delbruckii* is often used for the purpose—in some cases natural cultures are used. The pure cultures of selected strains give the best results. These are grown at 50° to 52° in small thermostat regulated tanks. At temperatures much below 50° butyric acid-forming organisms will develop; if the temperature is much higher than this, the lactic organisms are weakened and peptonization of the proteins is halted by destruction of proteases and coagulation of the barley and rye proteins. Diastase works more rapidly at temperatures of 60° to 65° than at 50° but gives a much higher proportion of maltose to dextrins at the lower temperature.

Lactic acid activates the proteases and checks or prevents the growth of undesirable bacteria such as *B. subtilis* during yeast growth or storage of the finished yeast. Success or failure depends as much upon the proper acidification of the mash as upon any other single factor.

Diastase rapidly converts the starch to maltose but peptonization of the proteins is an extremely slow process. Where three to four hours would suffice for the diastase eighteen to thirty should be allowed to permit the proteases to act. The yield of yeast depends upon the degree of protein hydrolysis. This long period of mashing can only be carried out by use of lactic acid cultures and accurate regulation of the temperature to favor the growth of the lactic organisms and to check the growth of other types.

During mashing the plastic mass of crushed malt and water becomes thin and watery because of conversion of starch to sugar. This change permits the liquid to be drained from the grain husks and the husks to be washed with water. The liquids so obtained are combined and filtered. The filtered liquid is of 11 to 14 degrees Balling and acid in flavor. In some factories the mash before straining or the liquid after straining is heated to a temperature sufficient to destroy the lactic organisms; in others this pasteurization is omitted.

The liquid now known as "wort" is cooled to the pitching (yeast inoculation) temperature of about 20° to 25°. The cooled liquid is transferred to large open vats, usually of 10,000 to 15,000 gallons capacity, either of wood or of enameled or otherwise protected steel. These are equipped with large copper cooling coils through which water may be circulated to prevent too great a rise in temperature of the wort from the heat of fermentation. Too high temperatures favor alcoholic fermentation with low yields of yeast. Compressed air is delivered to the liquid from perforated coils placed in the bottom of the vat. Vigorous aeration is used to favor the production of yeast and to limit alcohol formation.

Pure cultures of selected strains of yeast are employed for "pitching" purposes. This yeast starter is grown in sterilized wort in a small vat which can be kept free from undesirable organisms. This pure culture is replaced frequently by new starters propagated from pure cultures made by standard plating methods. This practice is necessary to insure purity of the yeast turned out by the large vats. Care must be taken to insure that the yeast used is true to type.

Fermentation and yeast growth in the large vats proceed for about fourteen to twenty-four hours. In some factories, the yeast is then allowed to settle. This takes place rapidly and almost completely if an agglomerating (granule-forming) type of yeast is used, very slowly if the yeast is of the fine-grained type. If settling can be accomplished, later operations are simplified, but settling is not essential to success.

The fermented liquid and yeast from the vats are passed through continuous centrifuges similar in design to milk centrifuges. A creamy suspension of yeast and liquid is obtained from one outlet and a liquid almost yeast-free from the other outlet of the centrifuge.

The creamy suspension of yeast is chilled by flowing over brine-cooled coils and flows to the filter supply tank. Chilling the yeast checks the growth of slime-forming and other harmful organisms.

The mixture of yeast and liquid is filter-pressed to remove excess of liquid. The pasty mass of yeast forming the filter press-cakes is usually mixed with a small amount of starch which gives a friable texture to the yeast. A small amount of vegetable oil is used in other plants for the same purpose. The yeast is next molded and cut into blocks of the desired shape and size. After wrapping it is held in cold storage until sold. At room temperatures the yeast liquefies through autolysis or becomes slimy from bacterial growth, moldy or weakened. Therefore, keeping the yeast at a low temperature is essential to longevity of the product.

The waste liquor from the centrifuges and filter press is distilled in a continuous still. The alcoholic distillate is diluted to about 10 per cent. alcohol and passed through charcoal or coke filled generators to produce distilled vinegar used principally by pickle manufacturers. The vinegar is sometimes used for aging, or, diluted to 5 per cent acetic acid, is sold for domestic use (See Division VI, Chapter IV).

Compressed yeast is also mixed with corn meal and compressed into cakes and dried at temperatures low enough not to affect seriously the yeast's vitality. The dried product is known and used in rural communities as "Magic Yeast," etc. This product makes it possible to obtain relatively pure and active yeast in isolated communities. Such yeast gives the best results if made into a thin batter or potato yeast-starter twenty-four hours before it is to be used in bread making.

The manufacture of compressed yeast is carefully controlled by chemical analysis and frequent microscopical examinations. Good compressed yeast should be slightly moist but not "sloppy;" the color should be creamy white, and it should show a fine fracture when broken. It should melt readily on the tongue. The flavor should be clean and free from any suggestion of butyric acid or putrefaction. It should show only a very few dead cells when the cells are mounted in a dilute solution of methylene blue. It will normally contain a few lactic bacteria but must be free from *B. subtilis* or putrefactive organisms. In a case that came to the writer's attention the yeast from a certain factory carried rather large numbers of *B. subtilis* which caused the yeast to become slimy. The infection was traced to the water used in the factory. In another case the yeast was found to be the cause of ropy bread because of its contamination with *B. mesentericus vulgatus*. The use of a mash highly acidified by growth of *B. delbruckii* or other lactic organisms will hold in check most "yeast disease" organisms.

Yeast as Food.—Yeast is very rich in protein and forms a readily assimilable human food or stock food. Breweries and distilleries produce very large quantities of yeast as a by-product. In the case of breweries, the yeast carries a considerable quantity of hop resins which impart a bitter taste to the product and render it unpalatable for human food. It is, however, fed successfully to stock with the spent grains. By suitable washing processes most of the bitter resins may be removed.

In addition to its nutritive value it is claimed by some that yeast taken internally or applied externally possesses remarkable healing properties for wounds, boils, pimples, etc. It is also claimed that yeast is rich in vitamins, the now popular growth-producing compounds. These contentions are, however, not yet fully substantiated by thorough investigations.

Yeast may be dried successfully at moderate temperatures, but tends to caramelize or darken at temperatures used for fruit drying. The dried product makes an excellent protein concentrate for stock or may be used satisfactorily in flavoring many dishes prepared in the household and is suitable for the preparation of soups. An extract may be made from yeast and concentrated to a syrupy consistency as is meat extract. The product has a flavor similar to that of meat extract and may be used for the same purposes.

Large quantities of yeast have been grown by factories in Germany primarily for stock food, using cheap molasses as a source of carbohydrate for yeast growth and ammonium sulphate as a cheap source of nitrogen for protein formation. The process offers a very quick method of producing protein from inorganic nitrogen. A combination of a bottom yeast of the *Strept. cerevisiæ* or *ellipsoideus* type and a rapidly growing film yeast of the *Mycoderma cerevisiæ* or *M. vini* has given the greatest yields of yeast per pound of raw material used. Dilute solutions yield relatively larger amounts of yeast than do more concentrated solutions.

Sugary solutions from the hydrolysis of wood may be used as a source of carbohydrate instead of molasses. In fact, a variety of waste products might be utilized in this manner. This method of producing cheap protein possesses great possibilities and will become an important industry, it is believed, as the world demand for food becomes more acute through increase of population.

BREAD*

Success in bread-making depends more upon the control of the various types of fermentation organisms used in the rising of the dough than upon any other step in the process. Generally, the rising of the dough, to which bread owes its lightness, is caused by yeast fermentation. The yeast is usually a strain of *Saccharomyces cerevisiæ.* It is normally accompanied by other organisms, notably, those of the lactic group. The character of the bread depends very largely upon these bacteria, its quality being improved by moderate growth and injured by excessive growth of these accessory organisms.

The yeast used in bread-making is produced by different methods, according to the custom of the various countries or regions. In the United States, compressed yeast prepared as described elsewhere in this chapter is used.

The compressed yeast is ordinarily employed in one of two general ways: By the "straight off" method and as "sponge" or "batter." In the former method, which is the one most commonly employed in bakeries, the yeast, flour, water, salt, sugar, or sugar substitute, and shortening (fat or oil) are mixed at once and permitted to rise. Milk or dried milk powder is often used to replace water wholly or in part, resulting in a loaf of higher nutritive value and richer flavor. In some cases, proprietary yeast foods are added with the other ingredients.

Salt is added to the dough to improve the flavor and to retard diastatic and bacterial activities; if unchecked, the diastase of the flour tends to soften the starch which gives a soggy loaf or too sweet a flavor.

Sugar is added to provide material for yeast fermentation—little of the sugar is used for multiplication of the cells for the reason that the time of rising of the dough is not sufficient to permit very great increase in number of the cells and the other yeast foods are not favorable to rapid growth. Sugar has been replaced in practically all modern bakeries by malt syrup. Malt syrup is made by concentrating to about 80° Brix in large copper vacuum pans a sweet wort made by the malting and mashing of barley or barley plus corn or rice grits. It is dark amber in color, possesses a strong malt flavor, and usually one to three per cent of lactic acid made by "souring of the mash" with pure cultures of lactic bacteria before concentration. Malt syrup possesses several distinct advantages over sugar. The lactic acid of the malt insures a "clean" fermentation in the dough, improves the flavor of the bread, causes the color of the crumb to become lighter, and improves the flavor. Unreported data obtained by the writer and experiments reported by A. Wahl* have shown the above statements to be warranted. Bread made with malt extract, instead of sugar, remains moist for a longer time probably because of the large amount of dextrin in the syrup. The malt sugar of the syrup ferments more readily than cane sugar and the syrup is rich in yeast food, two factors tending to reduce the amount of yeast necessary and to improve the character of the fermentation. It is doubtful whether there is sufficient diastase in malt syrup to convert so great an amount of starch into maltose as to affect the yeast fermentation, quality, and texture of the bread.

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* Prepared by W. V. Cruess.

It is probable that the shortening added to the dough does not affect the character of the fermentation; its principal functions are to improve the texture and the flavor of the loaf.

It has been found that the quality of the bread is improved and less yeast is required if a small amount of plaster of Paris, calcium sulphate, is added to the flour. Ammonium sulphate possesses a remarkable stimulating effect upon the yeast. If small quantities of this salt are used it all disappears during the rising of the dough. It is assumed that it is taken up by the yeast and converted into protein. One proprietary mixture used by bakers consists principally of a mixture of ammonium sulphate and plaster of Paris.

The amount of water added will vary with the strength of the flour, weak flours requiring less and giving, therefore, a smaller weight of bread than strong flours.

The flour and other ingredients are brought to a definite favorable temperature, 25° to 30°, before mixing with the yeast. The dough mixing room in modern plants is maintained at a constant temperature and high humidity by means of a thermostat and hygrometer controlled heating and ventilating system. The latter consists of a large fan, heating coils, air humidifying chamber, and air-distributing pipes. A high humidity is necessary to prevent the surface of the dough drying out during its stay in the mixing room. A constant temperature is necessary to insure that the bread will rise on "schedule" time if other conditions are carefully controlled. It is usual to mix the dough, knead the rising dough, "proof" the loaves, and bake the bread within a regular schedule of eight hours or less.

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* Wahl, A., Journal of Industrial and Engineering Chemistry, 1915, page 773.

Mixing of the ingredients must be thorough to insure uniform fermentation throughout the dough. Mixing also results in the inclusion of air with the dough which stimulates yeast growth and activity.

During the rising of the dough several changes take place. Carbon dioxide is formed and distends the dough by the formation of small bubbles of gas held by the gluten of the flour. Wheat flour is the only one possessing enough gluten to permit of this phenomenon. The proteins of the flour are to some extent peptonized by proteolytic enzymes of the flour, thus tending to reduce the harsh texture of the raw dough. Diastase is active to a slight extent and also tends to soften the texture of the dough. Lactic and other bacteria develop to an extent depending largely upon the time and upon the original number of such organisms present. Contrary to popular belief, lactic organisms are not the ones usually responsible for the "sour taste" of some breads, this undesirable flavor is usually due to butyric acid organisms.

Ordinarily the dough is allowed to rise in shallow wooden troughs in the dough mixing room. After it has risen sufficiently it is "cut down" and rekneaded. Often the cutting and rekneading are again repeated before the bread is molded. The cutting down prevents the bread rising too rapidly and permits a sufficiently long period of rising to insure the proper flavor and texture, and the frequent kneading results in the formation of a loaf of more uniform texture. Too much yeast causes the dough to develop large gas pockets or gas may escape from the dough from large bubbles, resulting in the breaking and slackening of the dough. Too little yeast results in too slow a fermentation with consequent increase in danger from growth of undesirable organisms.

The dough from the dough or "proof" room goes to mechanical molding machines which form the dough into loaves of the desired weight and deliver them to the proof box where the loaves are subjected to a temperature favorable to rapid fermentation. They may or may not be rekneaded before baking.

Baking is in many large plants accomplished in "traveling ovens" in which the loaves traverse the oven on heavy metallic conveyors, thus resulting in a great saving of labor and in standardization of baking time. The ovens are electrically controlled by thermo-regulators. Other bakeries use stationary ovens of various types and handle the bake pans by hand labor.

During baking, the gas pockets in the dough expand with the heat and increase the size of the loaf. The gluten is coagulated by the heat and thus retains the size and shape of the gas bubbles at the moment of coagulation. The yeast activity is for a short time stimulated but the cells are killed when the dough reaches 60°. Peptic activity is for a time stimulated, resulting in some softening of the gluten. Diastase increases in activity until 65° to 70° is reached and then decreases until the temperature is reached which destroys this enzyme. It hydrolyzes or gelatinizes some of the starch on the surface of the loaf. Heat dextrinizes part of the starch in this locality also, and the drying out of this layer of dextrinized starch gives the crust. The interior of the loaf probably does not reach a temperature much above 100° during baking because the evaporation of water maintains the dough at the boiling point of water. The carbon dioxide and alcohol formed during fermentation are driven off although some of the less volatile esters and organic acids formed by yeast or bacteria remain in the bread to give it its characteristic flavor.

Bread made by this method should have a flinty nut-brown crust which cracks when broken; the crumb should be porous, not soggy, free from large gas pockets, of even fine grained texture and elastic; the flavor should be clean and sweet and the color white or creamy white. It should not possess a disagreeable yeasty or butyric odor. A yeasty odor usually comes from stale yeast—fresh clean yeast can be used in very large quantities without imparting a yeasty odor or flavor.

In the "sponge dough" method commonly used by housewives and by some bakers a thin batter of yeast, flour, and other ingredients is prepared on the day before the dough is to be made. Potato flour or grated potato is often added to furnish yeast food. Malt syrup is used for the same purpose. Vigorous fermentation and probably considerable growth of yeast occurs in this batter which is softened through peptic and diastatic activity and considerable opportunity for bacterial growth is given. After about twenty-four hours standing it is mixed with flour and handled as described above in the straight dough method except that usually only one "cutting down" of the dough is employed. Bread made in this way usually possesses a more agreeable flavor and texture than bread made by the straight method and the bread remains fresh for a longer period. Where dry yeast cakes are used, the sponge method is to be preferred.

In many European countries a "sour dough" starter is used to leaven the loaf. According to the French method described by Boutroux* a little of the dough ready for baking is set aside and mixed with flour and water and permitted to stand four or five hours. This operation is repeated several times before the "leaven" is ready for mixing with the dough. The repeated addition of flour invigorates the yeast by supplying it with food, thus maintaining active yeast growth, which tends to discourage the growth of less desirable ferments. Fresh dough with about one-third its bulk of leaven is mixed for the final baking and fermentation is allowed to proceed again for a short time. In spite of the repeated additions of flour during preparation of the leaven there is a rapid growth of lactic and other bacteria resulting in the development of the acid and characteristic flavor of genuine French bread. The long fermentation results in considerable softening of the gluten, causing large holes to form in the dough, and in marked diastasic action which also affects the texture of the loaf. Imitation "French bread" as usually sold in the United States is made with compressed yeast and does not resemble true French bread in flavor.

Sour dough breads are common in Italy and southern Europe. Usually the fermentation is less carefully controlled than in France, imparting a more acid and to the American taste, a less agreeable flavor.

In some localities an impure natural yeast is prepared for bread making. Flour, hops, hot water, and ground malt, are mixed together, and allowed to stand until the diastase converts most of the starch to sugar. The sweet liquid is drawn off and allowed to ferment. The yeast so grown is known as "virgin barm." If a starter of yeast from a previous lot is added, the yeast is then termed "Parisian barm." The sweet liquid, according to Jago, is of about 14° Brix when freshly prepared. The hops serve to check the growth of undesirable bacteria. Nevertheless, the barm is a mixture of several yeast varieties and bacteria.

The fermented liquid and yeast are mixed with the flour in making up the dough.

The Mexican *tortilla* contains no leaven and is baked as soon as the flour, water and salt are mixed and thoroughly beaten. Similar breads are made in other primitive countries.

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* Boutroux, L. Le pain et la panification.

Salt-rising bread has been studied from the bacteriological standpoint by Kohman.* A starter is prepared by scalding corn meal with hot milk. Salt is added to the mixture. The mixture is kept in a warm place until in active fermentation and is then mixed with wheat flour and salt to give a dough of normal consistency. The organisms are of a spore-bearing type which readily survives a temperature of 75°, while *B. coli* and other undesirable organisms are eliminated. The organisms are furnished by the corn meal. These were purified by Kohman who found that they lost their gas-producing power when preserved in or on usual laboratory media, but retained their desirable properties including gas production if preserved in the dry state after mixing with a starchy material. The gases formed during salt rising bread fermentation were about two-thirds hydrogen and one-third carbon dioxide. Yeast is not of importance in the rising of this bread.

Bread is subject to a number of imperfections or diseases. Soggy bread may be due to a prolonged period of rising, use of insufficient yeast or poor yeast, use of too much water in proportion to the flour, or too weak flour, insufficient kneading or improper baking. Ropy or slimy bread is caused by spore-bearing organisms, usually *B. mesentericus vulgatus*, which live through the baking process. Either the yeast or the flour may be the source of infection. It is stated that bread becomes stale because of the migration of moisture from the crumb to the crust, leaving the crumb dry and crumbly and the crust pliable and soft instead of flinty and brittle. It is also due in part to the starch reverting from the semi-soluble form of the fresh loaf to its original harsh form. Molds of the mucor group sometimes develop quickly in a warm moist atmosphere.

Sour bread, the most dreaded of all imperfections, is usually due to the growth of butyric organisms. The clean sour flavor developed by lactic bacteria is not so objectionable, and is even preferred to the usual flavor of bakers' bread by many.

Micrococcus prodigiosus may develop on moist bread after long standing, with the formation of red spots. It seldom occurs, however, and is not considered harmful to health.

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* H. A. Kohman. Salt Rising Bread and Some Comparisons with Bread made by Yeast. Jour. Ind. and Eng. Chem., 1912, pp. 20, 100.

PRESERVATION OF VEGETABLES BY FERMENTATION*

Lactic acid is an excellent preservative and affords a very common means of preserving certain kinds of vegetables. The most familiar examples are sauerkraut and dill pickles. In some European countries many kinds of vegetables are preserved by lactic or "kraut" fermentation—this method replacing to a large extent preservation by canning. String beans, greens of all sorts, cauliflower, carrots, turnips, and beets are very satisfactorily preserved in this way; peas tend to become rancid in flavor.

Two general fermentation methods are used, dry-salting and brine For juicy vegetables, such as cabbage and greens, the dry-salting method is preferable; for large vegetables or vegetables of less water content fermentation in brine is advisable. Sufficient salt is sometimes used to prevent all fermentation.

In the preparation of sauerkraut the dry-salting process is used. The heads of cabbage are cored, the outer leaves are removed, and the remainder is cut into thin shreds. For each 100 pounds of cabbage 2½ pounds of salt is added and mixed with the cabbage in open wooden containers varying in size from a small barrel or crock for home use to tanks holding a thousand gallons or more in large factories. Pressure is applied to the mixture, by weights in the household and by screw or beam presses in factories. The osmotic action of the salt combined with the pressure forces the juice from the cabbage. In this juice a very vigorous gaseous and acid fermentation ensues in which the mannite and sugars of the cabbage are converted into lactic acid, acetic acid, alcohol, succinic acid (and in some cases butyric acid). Carbon dioxide, hydrogen, methane and various aromatic esters and other bodies are also formed. At the same time the protein is decomposed more or less. Without the addition of salt a putrefactive fermentation is apt to result; the salt to this degree acts as a governor of the types of fermentation that occur. It tends to favor lactic fermentation and check putrefaction. The fermented product owes its keeping qualities to the lactic acid formed during the fermentation period. The amount of acid formed is usually from 0.5 to 1.5 per cent.

Mycoderma vini and molds develop rapidly, especially in warm weather, on the surface of the fermented liquid and rapidly "devour" the lactic acid. This soon results in a reduction of the acidity to the point where putrefactive organisms may develop. These acid-destroying organisms are aerobic. Therefore, prevention of their growth consists simply in excluding air from the fermented material. Tanks or barrels may be filled with dilute brine and headed up; small containers are sealed with paraffin or vegetable oil to exclude air. The sealing of the container is not done until fermentation is complete. In warm weather complete fermentation will take place in eight to ten days; in cool weather two to four weeks is required. *Mycoderma* grows very rapidly in warm weather forming a heavy gray wrinkled film; in cold weather it develops very slowly or not at all; hence more precautions against its development must be taken during the summer months than during the fall or early spring.

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* Prepared by W. V. Cruess.

Weiss has isolated some sixty-five different species of bacteria from sauerkraut, most of which were indifferent or harmless. The writer can see no reason why *Bacillus botulinus* might not develop in cabbage or other vegetables during or following fermentation and suggests this as a good field for investigation by the various laboratories at present engaged in the investigation of *B. botulinus* in its relation to food preservation.

String beans and many varieties of greens may be preserved in the same manner as sauer kraut. The best results are obtained if a small amount of vinegar is added to the brine to favor lactic organisms and to discourage putrefactive forms.

In the manufacture of most pickles, the raw material (cucumbers, cauliflower, etc.) undergoes a lactic acid fermentation before receiving the final vinegar or other preservative solution or sauce. Normally, the cucumbers are first placed in a brine of about 45° salometer test (about 12 per cent sodium chloride) in open vats. As osmotic action and fermentation proceed, the brine becomes diluted by the vegetable juices; if the concentration falls much below 45° salometer, putrefaction and softening occur. An attempt is therefore made to increase the concentration to 60° salometer test (15 to 17 per cent sodium chloride) by progressive additions of salt. The vegetables are well covered by the brine. Vigorous lactic acid fermentation occurs in the brine and vegetables. *Mycoderma vini* and film-forming bacteria develop at the surface of the liquid but are usually not sufficiently active in the 60° brine to reduce appreciably the acidity. Cucumbers are often held a year or more in this way. Subsequent pickling operations consist in leaching out the excess salt in warm or hot water, firming the flesh with a dilute solution of alum and impregnation of the cucumbers with plain or spiced or sweetened vinegar.

Dill pickles are prepared by fermentation of cucumbers in a 40° to 45° brine (10 to 12 per cent sodium chloride) in closed barrels fitted with small vent holes for escape of gas. Dill and other herbs and spices are packed in the brine with the cucumbers before fermentation. Enough acid is formed to preserve the pickles indefinitely if air is excluded. Often the finished pickles are pasteurized in lacquered cans for domestic use. The high acidity renders the sterilization very easily accomplished.

Cauliflower, peppers, ears of sweet corn, and other vegetables have been held successfully in brines of 10 to 12 per cent salt (40° to 48° salometer test), although the products possess a distinct "kraut" flavor. If vegetables of any variety are mixed with 25 to 33 per cent of their weight of salt they may be preserved indefinitely without fermentation, provided they are sealed in barrels or jars or under paraffine to prevent evaporation of moisture. Corn and string beans are excellent so preserved. The process offers a home method of preserving vegetables without danger from botulism. Where dry salt can not be employed the vegetables may be packed in a saturated solution of salt. The salt preserved products must be soaked in water before use to remove excess of sodium chloride.

THE RELATION OF BACTERIA TO OLIVE PICKLING AND CANNING*

The green olive of commerce is a fermented product preserved by the lactic acid formed during the pickling process. The process used dates from antiquity and is the result of long years of experience and slow development.

Olives of full size but still immature are used. The Queen olive of commerce is principally of the Sevillano variety. The small green olives are usually of the Manzanillo variety, although other types are often used.

The fruit is first placed in a lye containing about 2 per cent of sodium hydroxide which is allowed to penetrate the fruit almost to the pit. The lye is then removed and replaced with cold water which is frequently changed until the fruit is practically free from lye. The lye hydrolyzes the amygdalin, a bitter principle of the olive.

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* Prepared by W. V. Cruess.

The olives are then placed in barrels and the barrels filled with a brine of 7 to 10 per cent. (28° to 40° salometer) salt solution and rolled into a warm room or the warm sunshine to undergo spontaneous fermentation. Many types of organisms develop but the high salt concentration favors the growth of lactic bacteria. Six weeks to two months is necessary for the fermentation. Air must be excluded to prevent the growth of acid-destroying organisms and to prevent browning of the color of the olives by oxidation. The finished olives are packed in glass containers without sterilization. The acidity of the fruit is relied upon to preserve the product. The characteristic flavor of green olives is due to fermentation. Often, however, various decompositions occur in the jar resulting in softening of the fruit and the development of a disagreeable flavor and odor. *B. coli* is a common offender in this regard. The writer believes that a much more healthful and sanitary product would be obtained if the bottled olives were pasteurized.

Ripe olives are packed extensively in California in cans. The finished product is dark brown or black in color and neutral or slightly alkaline in reaction. The usual process consists, first, in placing the ripe fruit in barrels or tanks filled with 5 to 7 per cent. brine. In these containers, a vigorous gaseous and lactic acid fermentation takes place for about two weeks and a film of yeast-like cells accumulates at the surface in many cases. Some factories omit this preliminary fermentation but those who employ it claim that it renders the olives firmer, porous, easily penetrated by the lye used in pickling, and of superior flavor. The writer's experience leads him to believe that the fermentation process has little merit and may be one source of infection of olives with *B. botulinus*.

The olives are treated, in the usual process, with dilute lye ¾ to 2 per cent sodium hydroxide for a period of time sufficient to permit the lye to penetrate the skin of the fruit and a short distance into the flesh. They are then exposed to the air or are submitted to a stream of compressed air in water to darken the color. The polyphenols of the olive flesh rapidly oxidize to a black color in the presence of air and dilute sodium hydroxide. The darkened fruit is given a second lye weaker than the first until the lye reaches the pit. This destroys the bitter principle. The lye is removed by repeated leaching of the fruit with water. In former practice it was then covered with a dilute brine of about 2 per cent sodium chloride which was gradually increased to 5 per cent. During this "salt curing" process of two to six weeks opportunity was afforded for a great variety of bacteria to develop. *B. coli* could be found very frequently. Often gaseous fermentation of the fruit developed or the surface of the olives became slimy through the growth of mold, bacteria, and even amœbæ or the fruit softened through putrefactive organisms. Most factories now eliminate or greatly shorten the brining process to avoid the bacterial changes noted above.

The olives are canned in a 3 to 5 per cent (12° to 20° salometer) salt solution and sterilized at 212° to 250°F., depending upon the factory. Recent investigations by the writer have shown that olives heated to 212°F. only in cans are not sterile but in practically all cases contain living spore-bearing organisms. Temperatures of 230° to 240°F. may be used for thirty to forty minutes without seriously injuring the quality of the fruit.

Recently several cases of botulism from commercially canned olives and one from green olives have been reported. Three factories were represented in these outbreaks. In all cases the olives have been processed in the cans at 212°F. only. No cases have occurred from this fruit sterilized under steam pressure. Cases have been most frequent from fruit canned in glass because of the difficulty of sterilizing glass containers under pressure without breakage. Dr. E. C. Dickson of Stanford University, Dr. K. F. Meyer of the University of California, and Dr. M. J. Rosenau are engaged upon an extensive and detailed study of the death temperatures and other properties of *B. botulinus* strains from food poisoning cases. Their work is under a grant from the National Canners Association, given as a direct result of the outbreaks from canned olives.

As now canned, ripe olives are safe because of effective sterilization methods.

Silage Fermentation*

The character of the changes brought about in silage varies with the material used in filling the silo and with the method of filling. Beet cosettes in silos undergo alcoholic and lactic fermentation, very large losses of sugar occurring through fermentation processes. Often 3 per cent or more of lactic acid is formed, beet pulp silage in this respect representing one of the most acid types of silage commonly produced. The proteins of the beets also undergo decomposition to a limited extent with consequent reduction of feeding value. Beet cosettes are now dried in most modern mills because of the large losses of feeding value in beet silage and because of the better keeping quality of the dried product.

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* Prepared by W. V. Cruess.

Pea vine and other highly nitrogenous materials tend to undergo putrefaction rather than lactic fermentation but make excellent silage when combined with corn or other starchy materials.

The fermentation of corn silage was first systematically studied by Burrill* who found that the hot fermentations sometimes encountered in silage were frequently induced by slow filling of the silo, thus permitting oxidizing thermophilic organisms to develop. Silage near the surface of the filled silo often reaches high temperatures for this same reason. Temperatures of 60° to 70° are not uncommon.

In the normal fermentations of silage, however, the temperature in the depths of the silo seldom exceeds 35°, permitting the growth of a varied microflora in which lactic organisms predominate. Acetic acid and propionic acids have been shown by Dox and Neidig of Iowa to be regular constituents of corn silage. Their results indicate that a small amount of butyric acid is to be found in normal silage and that this often increases in silage of poor quality. Formic acid was found in a few samples. Ethyl alcohol and propyl alcohol occur in determinable quantities and higher alcohols in traces. It is believed that the alcohols are formed as a result of bacterial rather than yeast fermentation as the yeasts found in silage are usually of the *Mycoderma* group rather than of the alcohol-forming types.

Silage resembles sauerkraut in many respects, although the fact that silage is less watery than sauerkraut modifies the character of the organisms and the intensity of the fermentation. Corn silage normally contains more than 1 per cent total acid.

So-called "sweet silage" may be caused in some cases by thermophilic fermentation which reaches a high enough temperature to destroy the lactic bacteria or in other cases by the use of material too low in sugar to afford enough of this compound for appreciable lactic acid formation. "Sweet silage" always contains an appreciable amount of acid.

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* Burrill, T. J., Biology of Silage. Bulletin 7. Illinois Station, 1889.

According to Hunter and Bushnell of the Kansas Station* four groups of organisms are responsible for the changes occurring in the silo: (1) the acid group, (2) the colon group, (3) yeasts, and (4) miscellaneous. They found that the most important acid formers belong to the bulgarian group. Recently the use of cultures of *B. bulgaricus* grown in milk as starters for silage fermentations has been advocated in farm journals. Whether this practice will prove successful and desirable remains to be seen, but it would certainly appear to be a logical procedure for the reason that the addition of lactic cultures to beet cosette silage has proved successful.

Probably a great deal of the gas formation in silage is due to members of the colon group.

Plant enzymes are of some importance in silage fermentation. Some of the starch of corn or corn stalks is converted into sugar by the plant diastase and the presence of a plant invertase which acts upon the cane sugar present has been demonstrated. However, it is believed that no appreciable proportion of the rise in temperature in the silo is due to these or other similar purely plant activities. It is held by most investigators that the important changes are due rather to bacterial activity.

Recently the work of Graham of Kentucky and others has brought to light in a startling manner the importance of *Bacillus botulinus* in relation to forage poisoning from silage and hay or straw. Because of the fact that hay or silage responsible for stock poisoning was usually moldy it was assumed that the mold was the responsible agent. Silage at the surface of the silo becomes moldy and alkaline in reaction and is considered by many stock men as poisonous because of the mold.

It has now been demonstrated, however, that the most common death-producing organism in silage is very similar if not identical with *B. botulinus.* Pearson as early as 1900 demonstrated the relation between a sporadic outbreak of forage poisoning and the poisonous quality of ensilage fed to the stock in question. Stonge and Buchanan of Iowa obtained positive results under conditions similar to the above. More recently Bush and Gridley of Illinois and Graham, Brueckner and Pontius of Kentucky, have shown *B. botulinus* or an organism closely resembling this organism to be responsible. Hart of the University of California has, late in 1919, studied several outbreaks of this character in California. He finds that the number of forage poisoning cases increases during the winter, probably because sufficient time has elapsed since the preceding harvest to permit formation of toxin in silage or hay.

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* Kansas Station Technical Bulletin 2 (1916).

To date, no definite method of preventing the growth of this organism in silage has been developed, although its growth is undoubtedly influenced by the composition of the silage and the character of the fermentations taking place in the product. It would appear logical to induce vigorous lactic fermentation in the silage in order to check the development of *B. botulinus*.

Hart of California found the two groups, namely, A and B strains, of the organism in forage poisoning cases and confirmed the work of others that the antitoxin from an A strain does not protect against the toxin of a B strain and vice versa. He has not had any notable success in preventing the death of animals already showing symptoms of botulism by the use of antitoxin but has obtained protection where the antitoxin is administered with the toxin or very shortly afterward. He has succeeded in immunizing horses to very heavy doses of the toxin and has thereby obtained the antitoxin in quantity.

The problems presented are extremely serious and important and well worthy the attention of all investigators in states which use the silo extensively. *B. botulinus* has of late intruded itself upon our attention in many ways and its control offers one of the most important problems confronting the bacteriologists of this country.

MALT SYRUPS*

Several types of malt syrup are produced commercially in the United States. The most common form is that used by bakeries in bread making. In preparing this syrup kiln dried malt is ground, mixed with water and mashed, first at a temperature of 50° to 52° to favor lactic fermentation, and later at 60° or higher to convert the remaining starch into maltose and dextrin. The resulting liquid is strained or filtered and is concentrated in vacuo to about 78° Balling. One of the most important constituents of the syrup is the 1 to 3 per cent. of lactic acid content. This is usually formed by selected strains of lactic organisms during the mashing process. In some factories, the lactic fermentation is carried out in a special tank separate from the general mash tank; this acidified liquid is then added to the saccharified liquid from the mash tank. This method greatly simplifies the mashing of the malt used for the main bulk of the syrup, making preliminary mashing at 50° to 52° unnecessary.

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* Prepared by W. V. Cruess.

"Malt glucose" or refined malt syrup is a colorless syrup resembling ordinary refined corn syrup in appearance but is sweeter and of richer flavor. It is usually prepared by saccharifying gelatinized corn or rice starch with barley malt; followed by decolorizing the sugary liquid with bone "coal" or vegetable decolorizing carbon and concentrating under a high vacuum. The use of starch with the barley malt reduces the amount of protein in the syrup and renders the liquid more easily decolorized than straight malt syrup.

Malt syrup containing in concentrated form an extract of hops as well as the maltose and other compounds from malt has been produced for small scale beer-making purposes or for export for beer making. It is made by mashing barley malt in the usual manner as for brewing, followed by boiling with hops and concentration in vacuo to a heavy syrup.

Partially refined malt syrup diluted to about 70° Brix makes a satisfactory table syrup.

Tobacco*

The curing of tobacco results in the evolution of considerable heat and rather profound changes in the composition, appearance, aroma, and flavor of the product. It was thought at one time that most of the changes were bacteriological but it now appears that enzymatic activities of the plant are also responsible for the transformations noted. From 12 to 40 per cent. of the dry matter of the leaves is lost in the various curing processes. The principal changes are the disappearance of starch and reducing sugar; a decrease in proteins, nicotine, pentosans, and malic acid; and an increase in citric acid. Ammonia is formed. After drying, the leaves are piled in masses, moistened, and allowed to undergo a fermentation which raises the temperature to 50° to 55°. In some cases the leaves are sprinkled with a solution containing sugar, honey, various aromatic substances, and sometimes alcohol and passed through another fermentation.

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* Prepared by W. V. Cruess.

The leaves are then usually tied up in bundles, partially dried, pressed in boxes where a final slow fermentation occurs. During this final fermentation nicotine decreases and nitrates are destroyed, while ammonia and sometimes butyric acid are formed.

It seems probable that the changes during the curing of tobacco are due in the first place to hydrolyzing, proteolytic, and oxidizing enzymes, and that these enzymatic changes are supplemented by the bacteria which destroy nitrates and produce ammonia. It is possible that these various factors account for variations in the characteristics of tobacco from the same source.

STARCH*

Starch is prepared from potatoes, corn, wheat, flour and other amylaceous substances. The present method of separation is by chemical means. Formerly it was accomplished by a complex fermentation.

For the fermentation method, the grain is soaked in water until soft, then ground and made into a paste which is allowed to ferment spontaneously or started with a leaven taken from a previous fermentation. Alcoholic, lactic and butyric microörganisms attack the sugar while others attack the gluten and cellulose. The fermentation lasts from twelve to twenty-five days according to the temperature and the resistance of the raw materials.

During fermentation, lactic and butyric acid, hydrogen sulphide, ammonia and carbon dioxide with traces of alcohol and acetic acid are produced. The process is stopped as soon as gas ceases to be given off and before putrid fermentation sets in. The starch which is set free settles to the bottom and is separated by decantation, washing and screening.

The washed starch is then allowed to settle for three or four days in water. The sediment that is formed consists of three layers, the top consisting principally of gluten, the second of gluten and starch and the bottom of comparatively pure starch. The layers are separated and the starch extracted from the two upper layers by repeated washings on inclined planes. The starch, owing to its higher specific gravity, remains near the lower parts of these planes.

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* Prepared by F. T. Bioletti.

SUGAR*

In the manufacture of sugar, microörganisms have no useful part but many forms may be injurious and cause serious losses. The juices of beets and sugar cane and the saccharine liquids obtained by presses or diffusion batteries form excellent media for the multiplication of many *Saccharomyces* and bacteria. They are controlled by cleanliness, rapidity of handling, and sterilization by heat. They are injurious by destroying sugar and thereby diminishing the yield, by inverting a portion of the saccharose and rendering the crystallization difficult and by forming gelatinous masses in the liquids.

Many of them are very resistant to heat. *S. zopfi* withstands a temperature of 66° for half an hour. *Streptococcus mesenterioides* forms chains of cocci surrounded by voluminous gelatinous sheaths which unite in zoögleic aggregations sometimes very troublesome in sugar factories. On account of its sheath it is very resistant to adverse conditions. It retains its vitality after drying for three and a half years. It is not killed by heating to 86° for five minutes and occurs in the hot liquids of the diffusion batteries.

TEA*

In the curing of black tea several fermentation processes occur. It is stated that yeasts and bacteria are of little importance in this curing process under normal conditions. The most important changes are brought about by enzymes and by a mold which converts the tannic acid of the tea to gallic acid. If the fermentation is too prolonged or moisture conditions improper, slime-forming bacteria develop, resulting in injury to or complete spoiling of the product.

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* Prepared by F. T. Bioletti.

CHAPTER VI*

MICROBIAL FOOD POISONING

General Considerations

Illness following the ingestion of food, more or less definitely ascribable to the food, has been long recognized. The Mosaic regulations in regard to foods forbidden to the Jews are evidently designed in part to avoid the occurrence of food poisoning. In recent times recognized instances of food poisoning have been sufficiently frequent to make the subject one of considerable importance, but there are undoubtedly many instances of actual food poisoning in which the causal relation of the food remains unrecognized or even unsuspected.

Food poisoning is usually suspected at once upon the occurrence of sudden acute illness in a number of people at the same time, after they have partaken in common of some particular food or foods. The causal relation is especially evident when, as sometimes happens, a large number of people are affected in the same way immediately after eating together at a banquet, not having been associated with each other either before or after the meal. When a smaller number of individuals is involved, the connection with food may be more obscure. For this reason most of the well-authenticated instances of food poisoning are instances in which many persons have been affected at the same time. Acute food poisonings involving only a few persons probably occur very frequently in the home, but they receive little public notice unless fatal, and are often dismissed as mere "errors in diet," or as "indigestion." A careful study of these cases is likely to be made only where there is suspicion of criminal poisoning, or some other practical end to be served by the investigation. Chronic forms of food poisoning are for obvious reasons very difficult to recognize with certainty and some of the forms of disease the causation of which has been ascribed to chronic food poisoning may eventually prove to be due to other causes. The subject is still in a very doubtful state.

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* Prepared by W. J. MacNeal.

Several different classes of food poisonings may be recognized according to the source of the poisonous substance.

The material of plants or animals may be naturally poisonous to man as a result of the physiological activity of their own living substance. Poison of this kind may be constantly present throughout the tissues, or it may be confined to certain parts, or it may occur only at particular times or seasons. Some instances of poisoning with fish and with mushrooms belong to this class, and possibly also some of the instances of poisoning with potatoes of high solanin content.

Plants and animals may feed upon substances not poisonous to themselves, and these substances may remain a constituent part of their bodies to poison man when consumed by him. Some poisonings with freshly killed game are considered to be of this nature.

Any food may contain foreign poison added to it by design or by accident, such for example as the salts of the various poisonous metals. The amount of tin or lead passing into solution in canned or tinned foods may conceivably be sufficient to cause poisoning, but there is no reliable evidence that it has ever occurred.

Animals may be infected with pathogenic bacteria or with other parasites capable of infecting man, and the use of food products from such animals may cause disease. Tuberculosis, trichinosis and tapeworm may be acquired in this way.

Any food may serve as the passive carrier of infectious agents, such as *B. typhosus*, and some foods may even favor the multiplication of pathogenic bacteria gaining access to them.

A food may undergo chemical changes due to microörganisms incapable of infecting man, resulting in the production of poisonous substances in the food. Undoubtedly the great majority of instances of food poisonings belong in this class. The bacteria causing these changes have been designated as pathogenic saprophytes.

The last three classes comprise the microbial food poisonings, and these are the kinds of food poisoning with which we are at present more particularly concerned.

INFECTIONS OF FOOD-PRODUCING ANIMALS TRANSMISSIBLE TO MAN

Animals dead of infectious diseases or slaughtered in the last stages of disease are not ordinarily used for food, nor is the milk of such animals ordinarily considered wholesome. This custom is certainly an ancient one, and is doubtless founded upon observation of unfavorable results following the consumption of such food. Exact knowledge of the nature of the diseases transmitted in this way is a more modern development, and this more exact knowledge is now being applied to some extent through food-inspection regulations to prevent the transmission of such diseases.

Tuberculosis of cattle has been shown by Smith to be due to a germ somewhat different from that causing the ordinary human tuberculosis, and this discovery has called into question the necessity of avoiding the use of food products from tuberculous animals. After a considerable amount of controversy it may now be regarded as definitely established that the bovine type of tubercle bacillus is capable of infecting man, and that a very considerable proportion of cases of tuberculosis in children is due to this type of organism, the infection probably arising through the use of milk from tuberculous animals. Anthrax, glanders, actinomycosis and acute enteritis of animals are transmissible to man. Food products from animals afflicted with these diseases should not be used until they have been passed upon by competent authority. Further information concerning them will be found in the sections dealing with these particular diseases.

The human disease known as septic sore throat may be due to infection with streptococci present in cow's milk. Careful investigations by various independent workers have shown that these virulent streptococci may be derived from infected udders of the cows and the same studies indicate that the infection in the cow may be derived primarily from human sources. In some rare instances the disease in the cow has been traced to the introduction of a milking tube into the teat canal to facilitate the flow of milk and the evidence against the practice is sufficient to warrant its prohibition.

In this connection it may be mentioned that some of the animal parasites, especially trichinæ and various sorts of tapeworms, gain access to the human body with the food. Thorough cooking usually serves to kill these parasites, as well as the pathogenic bacteria, but ordinary cooking should not be too implicitly relied upon to accomplish this result.

HUMAN INFECTIONS TRANSMITTED IN FOOD

Food may serve as the passive carrier of the germs of any human infectious disease capable of indirect transmission upon dead material. In some foods, especially milk, these infectious agents may actually multiply. Typhoid fever, diphtheria and scarlet fever appear to be rather frequently disseminated through the agency of food, and paratyphoid fever seems to be commonly transmitted in this way. Especial precautions are advisable to prevent persons afflicted with dangerously communicable diseases and those who are chronic germ-carriers from engaging or continuing in occupations concerned with the immediate preparation of food for consumption, particularly such occupations as milk production and handling, market-dairying, cooking and serving food. Numerous serious epidemics have been traced to such sources in recent years. The history of Mary Mallon (Typhoid Mary)* has become popular knowledge but instances of similar spread of infection are but too common.

FOOD POISONING DUE TO THE GROWTH OF SAPROPHYTIC BACTERIA IN THE FOOD

Most food poisonings are due to food derived from perfectly healthy and wholesome animals or plants, which has subsequently undergone some bacterial decomposition giving rise to poisonous products. Our knowledge of the specific causes of the poisonous changes is, however, very incomplete, and on account of the difficult nature of investigation in this field, some of the conclusions reached by careful men are still open to question. The bacteria which have been most frequently identified with various epidemics of food poisoning are the following: *B. enteritidis* in meat poisoning; *B. botulinus* in meat, sausage and vegetable poisoning; *B. paratyphosus* in poisoning with meat, chicken, shellfish, and vegetables; *B. coli* in cheese poisoning and in milk poisoning; *B. vulgaris* in meat and in vegetable food poisonings. Doubtless other microörganisms, as yet unrecognized, play an important part in many food poisonings, and there is reason to believe that some of these important unknown forms are anaerobic bacteria.

POISONOUS MEAT AND SAUSAGE.—The flesh of a healthy animal is ordinarily free from bacteria at the time of slaughter, and bacterial changes must begin at the surfaces of the pieces of meat and gradually extend inward. In diseased animals, bacteria more frequently circulate in the blood, and the flesh may be contaminated throughout when the animal dies of the disease or when it is slaughtered, not only with the specific germs of the disease but also with bacteria derived from the intestinal tract of the animal. It is a matter of observation that the flesh of diseased animals is more liable to undergo early putrefactive and poisonous changes than that derived from healthy animals. Hashed meat is, of course, much more prone to bacterial decomposition, because in it the bacteria have become well distributed throughout the mass, and ideal conditions are provided for the development of anaerobic as well as aerobic bacteria. Minced chicken and chicken pie appear to be very frequent sources of acute poisoning in the United States, and epidemics of sausage poisoning have repeatedly occurred, especially in Germany. The bacteria found to be concerned in these instances have been *B. enteritidis*, *B. paratyphosus*, *B. coli*, and *B. botulinus*. Some of these poisons, as for example the toxin of *B. botulinus*, are rendered inert by boiling, but occasionally bacterial poisons which are not destroyed by such high temperatures may be present in food.* Moreover, meat rendered poisonous by these bacteria may show no evidence of putrefaction. *B.* (*Proteus*) *vulgaris* has also been found in some samples of poisonous meat, and this finding is usually associated with definite evidence of putrefaction.

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* Soper, George A.: Typhoid Mary. The Military Surgeon, July, 1919, Vol. 45, pages 1–15.

The symptoms of meat poisoning are usually those of acute gastro-enteritis,—vomiting, cramps, and diarrhœa. The patients often recover very quickly, but occasionally the illness is rapidly fatal, or it may merge into a subacute form resembling or identical with paratyphoid fever. In those instances of poisoning due to the presence of *B. botulinus* the symptoms are of a different kind, consisting almost solely of nervous disturbances, secretory and motor paralyses, without fever, resembling in many respects poisoning with atropin. In this form of meat poisoning the death rate is relatively high, about 40 per cent of the cases ending fatally.

Fish poisoning is of two general kinds, that due to poisons natural to the fish, and that due to poisons formed by bacterial activity in the flesh of the fish. Blanchard has applied the Spanish name "Siguatera" to the first kind and the term "Botulism" to the second. In the

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* Smith and Ten Broeck. Jour. Med. Research, 1915, 31, pages 523–546.

Japanese fish of the genus Tetrodon the roe is poisonous, giving rise to severe gastro-intestinal irritation and convulsions. The remainder of the fish is not poisonous. In some other fishes the sexual glands are poisonous during the spawning season; others are provided with special poison glands connected with protective spines or barbs. These are examples of poisons natural to fish. Bacterial poisons are likely to be formed in any kind of fish, given the suitable conditions, and thus give rise to the kind of fish poisoning designated as botulism. Cases of this kind have resulted from eating (spoiled) canned salmon and sardines. Poisoning may also result from eating diseased fish, the effects being due to poisons elaborated by the infecting bacteria in the body of the fish before consumption. This appears to be a rather common form of fish poisoning in Russia. *B. paratyphosus* has been isolated from some poisonous fish, and certain toxicogenic anaerobes have been found in others.

POISONING WITH SHELL-FISH is so well recognized that this form of food is not customarily used at all during the warmer part of the year, May to August inclusive, the months without an *r* in their names. *Shell-fish* may serve as carriers of human infectious diseases, such as typhoid fever; they may be poisonous on account of actual disease or through serious contamination due to living in dirty water; or they may be poisonous because of decomposition which has taken place after removal from the water. According to the symptoms produced, there appear to be at least three distinct varieties of shell-fish poisoning, one a purely gastro-intestinal disorder, the second an involvement of the nervous system with itching skin eruption and convulsions, and a third type resembling very closely alcoholic intoxication. The exact nature of the microbic agents concerned in these different types of poisoning is unknown. It is pretty well established, however, that the poisonous character of shell-fish is due either to their living for some time in dirty water, or to their too long preservation, especially at high temperature, after removal from the water.

MILK, ICE-CREAM AND CHEESE sometimes give rise to poisoning, and although these instances are small in number in comparison with the enormous amount of milk and milk products consumed, yet in the aggregate they are numerous. That many human infections may be transmitted by milk has already been pointed out. In summer, milk is undoubtedly a great factor in the infant morbidity and mortality, and this poisonous action is largely due to bacterial changes in the milk. Extraordinary precautions are therefore essential in the production and care of milk to be used as food for children, particularly during the warmer season of the year. Severe poisoning of adults with milk, ice-cream, or cheese, is relatively less frequent. Cases which have been studied have been traced to the development of *B. coli* or *B. paratyphosus* in these foods. There is some evidence that other bacteria, probably strict anaerobes, are also sometimes concerned. Strict cleanliness, proper refrigeration, and pasteurization of milk of uncertain character may usually be relied upon to prevent milk poisoning. Ice-cream should be made only from wholesome materials and with due regard to cleanliness in making it. The causes of serious cheese poisoning are not definitely known, but such poisoning may be avoided, to a large extent at least, by using only standard varieties of cheese of the proper odor and flavor.

VEGETABLE FOOD POISONING, in an acute form, has followed the use of sprouting and partly decomposed potatoes, and also various canned vegetables, particularly those of high protein content, such as beans. The large majority and possibly all of these cases are due to decomposition changes in the foods, *B. botulinus* and *B. proteus* appearing to be the microbes most frequently concerned.

SPECIFIC DISEASES DUE TO FOOD POISONING

BOTULISM AND BACILLUS BOTULINUS.—Perhaps the most serious and most rapidly fatal of all the food poisonings is botulism, a disorder caused by a true bacterial toxin formed in food previous to its ingestion, by the growth of a specific anaerobic organism, *Bacillus botulinus.* The earliest recognized cases of this disease were observed in Würtemberg, Germany, and followed the eating of sausages, hence the name botulism or sausage poisoning. Mayer has recorded 812 cases of botulism in Germany up to 1913, with 365 deaths. Dickson* has collected records of 64 cases in the United States from 1894 to 1918, of which 41 resulted in death. The mortality in this series has been, therefore, 64 per cent. It seems certain that only a small proportion of actual existing cases has been recognized and that milder outbreaks of the poisoning, especially, have escaped record. In one outbreak the mortality was 100 per cent. and in another only 8.3 per cent.

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* Dickson, E. C.: Botulism. Monograph No. 8 of the Rockefeller Institute for Medical Research, 1918, p. 51.

The first symptoms of botulism appear, as a rule, in from eight to one hundred and twenty hours after the poisonous food has been taken. In exceptional instances they may appear as early as two hours after the meal or be delayed until the ninth or tenth day. In most instances the first symptoms are referable to paralysis of motor or secretory nerves. Double vision due to paralysis of the external recti or even of all the extra-ocular muscles, dilated and sluggish pupils, difficulty in swallowing, dryness of the mouth, nausea and vomiting, accumulation of mucus in the paralyzed pharynx resulting in paroxysms of choking, persistent constipation, rapidly progressive weakness, with undisturbed sensation and clear mentality, are the most characteristic manifestations of the disease. Disturbance of vision is often the initial symptom. Less frequently the illness begins with vomiting. Usually the temperature remains subnormal but fever is present in some instances, possibly on account of complicating bronchopneumonia or other terminal infection. The following graphic description of the fully developed disease is quoted from Dickson's monograph (page 46): "The general appearance of the patient is most distressing. The extreme muscular weakness, the anxiety and the utter helplessness, the difficulty in swallowing, the attacks of strangling, the struggle for breath, and the unsuccessful attempts to articulate constitute a clinical picture which, when once observed, can never be forgotten. The face is usually pale, but in the early stages may be congested. There may be normal appetite and excessive thirst, but the patient is afraid to try to swallow. At times the strangling spells are so severe that there is incontinence of urine, and the accumulation of thick, tenacious mucus in the pharynx is a constant source of annoyance. The fact that the patient remains in full possession of his mental powers and can realize the seriousness of his condition only adds to the distressing character of the situation."

At autopsy, congestion of the central nervous system is constantly found and, in nearly every case, thrombosis of the meningeal vessels as well as the blood vessels in various other organs. The ganglion cells are well preserved in many cases although some of the earlier observers recorded disturbances of the Nissl granules and displacement and distortion of the nucleus of the motor cells. The cerebrospinal fluid contained 80 cells per cu. mm. in one case during life. The differential diagnosis of a single case of the disease in the absence of history suggesting food poisoning presents great difficulties and there is good reason to believe that many instances of botulism fail to be recognized during life or at autopsy.

Various animals are subject to botulism. It has been established that horses, mules and domestic fowl suffer and die from the disease naturally. Dogs, cats, guinea-pigs and rabbits are susceptible to the experimental disease. Cattle and chickens, though susceptible, seem distinctly more resistant than horses. Graham and his associates have brought forward convincing evidence to prove that at least some examples of "Forage poisoning" and "Ensilage poisoning" in domestic animals are actually due to toxin of *B. botulinus* produced in the feed. In these animals paralysis and muscular weakness are the prominent manifestations. The disease has been designated as limber neck in chickens and as cerebro-spinal meningitis, staggers or blind staggers in horses. The proof that the disease is botulism rests upon the isolation of *B. botulinus* from the food which gave rise to the poisoning, or the effective protection of experimental animals against the poison in the food by administration of specific botulinus antitoxin to them while unprotected control animals are fatally poisoned, or by the successful results of both these experimental procedures.

Bacillus botulinus is a large rod 0.9 to 1.2μ wide and 4 to 6μ long, single, in pairs or in longer threads when growth conditions are unfavorable. The spore is oval and causes enlargement of the cell. It is usually terminal or near one end, but may be central. The bacillus is slightly motile, possesses 4 to 8 flagella and is Gram-positive. It is a strict anaerobe, although, like other anaerobes, capable of active growth in symbiosis with aerobic bacteria in the presence of air. Glucose and salt in dilute solution favor growth but a concentration of 6 per cent. sodium chloride inhibits development. The optimum temperature for growth is about 28°C. Below 16° and above 37°C. growth is slight. The spores, according to Van Ermengem, are killed in a half hour at 80°C. and by boiling for five minutes. More recent careful tests by Burke have shown that this earlier work cannot be relied upon and that there is considerable variability in the resistance of the spores produced on different culture media. She found that some of the spores resist boiling water for two hours and the spores of one strain resisted heat in the autoclave at 5 pounds pressure for ten minutes. The importance of these observations in relation to canned food is obvious.

The poison of *B. botulinus* is a true bacterial toxin, which in its potency belongs in a class with the toxin of diphtheria and tetanus. Dickson has produced a crude toxin of which 0.0001 c.c. killed a guinea-pig within twenty-four hours. Unlike these other toxins, however, botulin (the botulinus toxin) is actively poisonous when swallowed with food as well as when injected into the tissues. An antitoxic serum has been produced by immunization of goats. This serum has considerable value in preventing the disease but little value in treatment after the symptoms have appeared. The analogy with tetanus is evident. Botulin, like the tetanus toxin, has a strong avidity for nerve tissue. The toxin loses strength slowly when heated at 56°C. and is rendered harmless by heating at 80°C. for thirty minutes or by boiling for five minutes.

Botulism has long been known as a form of meat poisoning and it has also been known that vegetable foods of high protein content, such as beans, might give rise to this poisoning. The clinical and experimental observations of Dickson, Graham and his associates have called attention to the possible production of botulinus toxin in various vegetable foods, including canned corn, asparagus, spinach, apricots and peaches as well as oats, hay and ensilage. The virulence of the poison is such that a mere taste of the tainted food is sufficient to cause serious illness and the swallowing of a single spoonful has caused fatal poisoning.

Botulism may be caused, therefore, not only by the consumption of meats and meat products but also of vegetable foods and it is especially important at this time to emphasize the danger in canned foods, especially in home canned foods. So far, botulism has been traced to commercial canned foods very rarely indeed but the methods used at home, especially the cold-pack method, may be quite inadequate to destroy spores of *B. botulinus* if they have gained access to the food. The canning of vegetables which are not sound and clean is an important source of danger. Even in commercial canning with standardized methods and control it is doubtful whether the heating is adequate to destroy spores of *B. botulinus*. Weinzirl* has found viable spores of aerobic bacteria in marketable commercial canned foods and he regards the absence of oxygen as essential to the preservation of such canned foods. If the spores of *B. botulinus* are as resistant as appears from the studies of Burke, then we must expect botulism from commercial canned foods unless *B. botulinus* is excluded by critical selection of sound materials and cleanliness throughout the canning process.

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* Weinzirl, J. The bacteriology of canned foods. Journal of Med. Rsch., Jan., 1919. Vol. 39, No. 3, p. 348–413.

Signs of spoilage due to *B. botulinus* may not be evident at all. Careful examination will often reveal gas bubbles in the container, an odor suggesting rancid cheese and a mushy disintegrated appearance of the solid contents. Canned foods showing any of these signs should never be tasted until they have been cooked again. Indeed, Dickson concludes that all home canned foods should be cooked again before being eaten. Boiling for five minutes just before serving the food practically removes the danger of botulism.

Ergotism is a disease characterized by cachexia, gangrene and convulsions. It is caused by eating the fungus, *Claviceps purpurea*, which grows as a parasite upon rye. The grain of this parasite has a considerable commercial (medicinal) value sufficient to pay for its separation from rye where it occurs, so there is little economic excuse for food poisoning from this cause.

Beriberi or *kakke* is an acute or chronic nervous disorder which has been observed especially in the Orient, Japan and the Philippine Islands, although it has also been found in Brazil, in Labrador and rather frequently among sailors after long sea voyages. At one time the disease was ascribed to the use of fish as food, later to the use of rice. Modern studies, especially those of Chamberlain, Vedder and their associates in the Philippine Islands, have shown that beriberi may be prevented by including beans, unpolished rice or rice hulls in sufficient quantity in the diet and furthermore that those already afflicted with the disease usually recover completely when given these foods or when treated with an alcoholic extract of rice polishings. The curative principle of rice polishings has been studied by Funk who has named it vitamine. He ascribes the causation of beriberi to a lack of this supposedly necessary vitamine in the food and this theory has been very favorably received. It must be acknowledged, however, that the etiology of beriberi is still not convincingly proven. The discovery of a remedy which eradicates a given disease is not sufficient to prove that the lack of this particular therapeutic agent is the essential cause of the disease.

Pellagra is a cachexia, characterized by a definite sort of skin eruption, which has been ascribed to the use of maize (Indian corn) as food. This disease is discussed in a separate section (page 865).

THE CHEMICAL NATURE OF FOOD POISONS

The poisonous substances in foods are for the most part of the same nature as the poisons of the pathogenic bacteria. The simplest in structure of these poisons belong to the alkaloidal substances, substituted ammonia and ammonium compounds, called *ptomains* (page 241). Several of these have been prepared in a pure state, for example, mytilotoxin ($C_6H_{15}NO_2$) from poisonous shell-fish and neurin ($C_2H_3 \cdot N(CH_3)_3OH$) from putrefied horse, beef, and human flesh. Although ptomains undoubtedly occur at times in poisonous foods, they are not now considered of so much importance in food poisoning as formerly, for in the majority of samples of poisonous food the search for ptomains has been in vain. The poisonous effects are believed rather to be due for the most part to much more complex bodies resulting from the earliest analytic changes in the food protein, or else to bodies built up by actual synthesis by the bacteria. Such substances are classed with the toxic proteins and the true toxins. Their chemical composition and structure are not definitely known.

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CHAPTER VII*

MICROORGANISMS OF THE DIGESTIVE TRACT

INTRODUCTION

The digestive tube of the vertebrate animal is in communication with the external world and is the passageway for a great variety of materials constituting the food of the animal. This food brings with it various sorts of microbes, at times in considerable numbers. Within the digestive tube the food is more or less completely resolved by the processes of digestion into soluble nutritive split products, which furnish an excellent medium for microbic development. It is not surprising, therefore, that there is an enormous multiplication of microörganisms within the intestine, both in health and disease, and that this multiplication is most active during the digestion of the food.

MICROÖRGANISMS OF CERTAIN PORTIONS OF THE ALIMENTARY CANAL

The entire digestive tract is free from microbes during normal intrauterine life. After birth the canal is quickly invaded by bacteria, chiefly through the mouth and nose, but to a lesser extent also through the anal orifice. In the mouth, pharynx and intestine, some of these invaders establish themselves to remain throughout the life of the individual host. The species of microbes present and the numerical proportions of the different species of normal buccal and intestinal microörganisms vary somewhat with the age of the host and the character of his food. They are also considerably disturbed sometimes by the entrance and multiplication of pathogenic germs, giving rise to disease in their host, such as *Oidium albicans* in the mouth or the cholera vibrio in the intestine.

MICROÖRGANISMS OF THE MOUTH.—The buccal cavity presents conditions of temperature, moisture, chemical reaction and a variety of food substances in its various parts, which are very favorable to the growth of many microbic species. Aerobic, facultative and anaerobic forms are found and the species are very numerous. Miller, in a few weeks, was able to isolate more than a hundred different kinds of bacteria from the mouth. Many of these are doubtless only transient residents, having gained entrance with food, water or air.

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* Prepared by W. J. MacNeal.

Among the almost constant inhabitants of the mouth may be mentioned the streptococci, both the variety which produces a green color on bloodagar, the *Strept. salivarius* or *Strept. viridans*, and the hemolytic variety, *Strept. hæmolyticus;* the *M. pyogenes var. aureus* and *albus;* the *Iodocóccus magnus* and *Iodococcus parvus* of Miller, which may be cultivated upon a sugar-starch gelatin-agar medium and are stained blue by iodine; two or three species of spirilla, described by Miller, which may be cultivated with some difficulty upon ordinary nutrient agar; *B. fusiformis* of Vincent, which may be cultivated as a strict anaerobe in media containing blood serum or ascitic fluid; *B. maximus* (*buccalis*) of Miller, a bacillus forming threads 0.5 to 1.5μ wide and 20μ or more in length, cultivable upon maltose agar or potato gelatin; *Leptothrix buccalis*, a slender unbranched filament, which may be brought to development on ordinary media, with some difficulty.

Even more definitely characteristic mouth bacteria are those which are found in every human mouth (except in very young children) and which are not cultivable in artificial media at all or only under special artificial conditions never met with in nature. Among these forms may be mentioned the *Iodococcus vaginatus*, an encapsulated organism which may be stained blue by Lugol's solution acidified by addition of lactic acid; the *Sp. sputigenum*, which is found especially at the inflamed margin of the gums; the *Spirochæta buccalis*, *Spirochæta media, Spirochæta microdentium* and *macrodentium*, organisms which are found in the mucus about the teeth, but are especially numerous on denuded areas or in abscess cavities of the gums or in carious teeth. The spirochetes of the mouth have been successfully cultivated by anaerobic methods in serum and in ascitic fluid by several investigators, notably by Noguchi.*

The amœba of the mouth, *Entamœba* (*Endamœba*) *buccalis*, may be found in nearly every individual in the deposits between the teeth and especially in carious teeth. The cell is 6 to 32μ in diameter, actively motile, with few lobose pseudopodia. The nucleus of the living amœba is visible. Its food apparently consists of bacteria and the bodies of leucocytes. It does not appear to penetrate living tissue. Other mouth amœbæ have been described. Whether they really belong to species distinct from *Entamœba buccalis* is questionable. Recently it has been claimed that the amœbæ of the mouth bear a causal relation to *pyorrhea alveolaris*, but the claim has not been convincingly proven.

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* Noguchi, H.: Jour. Exp. Med., 1912, XV, 81.

The various characteristic buccal microörganisms are found in particular parts of the mouth and their numbers vary considerably according to the cleanliness of the mouth and teeth, presence or absence of denuded areas, ulcers, sinuses or carious teeth. The iodine-staining varieties are especially abundant between the teeth and upon starchy food residues. The spirochetes, on the other hand, are more abundant in the serum exuding from denuded areas and in pus cavities. *B. fusiformis* (Vincent) is often found in normal buccal mucus but it is especially abundant in the necrotic ulcers of the tonsil in the disease known as Vincent's angina, in which situation it is always associated with numerous spirochetes.

Some of the members of the normal mouth flora occasionally play definite pathogenic rôles. There can be little doubt that the starch-fermenting forms produce acid, thus attacking the mineral matter of the teeth and favoring dental caries. The pathogenic rôle of *Strept. viridans*, when it penetrates into carious teeth, causing root abscess and, probably by metastasis from this focus, giving rise to arthritis and endocarditis, is indicated by a mass of circumstantial and experimental evidence which is well nigh convincing. The frequently serious nature of infections with the hemolytic streptococcus are well known. Doubtless members of this variety of streptococcus in the mouth are ready to acquire virulence whenever lowered resistance of the host presents a favorable opportunity for them to invade the tonsils, the pharyngeal mucous membrane, the Eustachian tube and middle ear, not to mention more distant parts of the body.

Certain very specific pathogenic microörganisms are found in the mouth and pharynx from time to time and they sometimes produce lesions there. *Spirochæta pallida* is especially abundant in the buccal and pharyngeal lesions of secondary syphilis. The tubercle bacillus is expectorated through the mouth in open pulmonary tuberculosis. The pneumococcus is often found in the mouth and pharynx, even in health and is especially numerous and virulent in cases of lobar pneumonia. The influenza bacillus and *Bact. diphtheriæ* are also occasionally found in the throats of healthy persons as well as of those suffering from the diseases to which they give rise.

MICROÖRGANISMS OF THE STOMACH.—The microbic flora of the healthy stomach consists almost exclusively of organisms swallowed. The gastric juice restrains bacterial multiplication and kills a large majority of the bacteria which enter the stomach. In diseased conditions the absence or reduced concentration of the hydrochloric acid may permit the multiplication of yeasts, of large lactic-acid bacilli (Boas-Oppler bacilli), of encapsulated cocci (*Sarcina ventriculi*), or even of flagellate protozoa, such as *Lamblia* and *Trichomonas*.

MICROÖRGANISMS OF INTESTINE.—The duodenum receives from the healthy stomach relatively few living bacteria. The secretions of the liver, pancreas and of the duodenal wall are very free from bacteria and they tend to flush out the duodenum. In health this portion of the intestine is quite free* from living bacteria in the intervals when food is absent and it contains relatively few bacteria during digestion. Among the living microörganisms most frequently found here are Gram-positive cocci which fail to liquefy gelatin. *B. coli* is uncommon. In spite of the negative results of culture work upon duodenal juice, it is always possible to see with the microscope abundant bacterial cells in it. These are probably dead.

From the upper end of the jejunum to the ileocecal valve, the number of bacteria in the small intestine progressively increases. In the intervals when food is absent, even these portions of the small intestine tend to free themselves from bacteria, in part, probably, because they are continually flushed out by the intestinal secretion, but probably in part also, as has been maintained by Kohlbrugge,† because of a definite bactericidal property of the intestinal mucous membrane. However this may be, it is certain that living organisms of the *B. coli* group and various streptococci are commonly found in intestinal contents taken from the jejunum or ileum at operation or at autopsy and that these organisms are quite numerous in the material discharged from the lower end of the small intestine in cases of ileocecal fistula.‡ The relative abundance of the different kinds of bacteria may be altered by changing the character of the diet, a fact of importance in the treatment of intestinal infections.

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* MacNeal and Chace, Arch. Int. Med., 1913, XII, 178.

† Kohlbrugge, Centrabl. f. Bakt. Abt. I, 1901, XXIX, 571; *ibid.*, 1901, XXX, 10; *ibid.*, 1901, XXX, 70.

‡ Macfayden, Nencki und Sieber, Arch. f. Exp. Path. und Pharm., 1891, XXXIII, 311.

In the cæcum there is a sudden enlargement of the lumen of the intestinal canal and a consequent retardation of the movement of the intestinal contents. The blind pouch also favors stagnation. In this region the whole intestinal contents usually acquire a chemical reaction neutral or alkaline to litmus. All these factors favor the enormous multiplication of bacteria. Indeed, the cæcum and the remaining large intestine constitute the great bacterial incubator of the healthy body. Here *B. coli* multiplies enormously; the strict anaerobes, *Bact. Welchii* and *B. edematis* flourish under most favorable conditions. Various streptococci, staphylococci and spirochetes multiply either in the food residues or in the intestinal secretions. An easily digested mixed diet favors the facultative anaerobes, while excessive feeding of starchy foods and of meat leads to an overgrowth of the strict anaerobes, especially those of the *Bact. Welchii* group. Many of these bacteria will then be found to stain blue with iodine, giving the so-called granulose reaction. A milk diet, especially if limited in amount and well digested by the individual, favors the micro-aerophilic *B. bifidus* of Tissier, the organism which is dominant in the fæces of the healthy breast-fed infant and occasionally very abundant even in adults.

In the lower portions of the large intestine, as a result of progressive absorption from the contents of the bowel, there is a concentration and overcrowding of the bacteria which have developed at higher levels. The vast majority of them die and these dead cells, together with the still abundant living microörganisms, make up about a third of the substance of the fæces. The fæces are composed of rejected food residues, residues of intestinal secretions, of bile and pancreatic juice and abundant microörganisms, some of the latter still actively multiplying, but the majority of them dead and in various stages of disintegration.

THE MICROÖRGANISMS OF THE FÆCES.—The microörganisms of the fæces represent the end result of the progressive multiplication or disintegration, or both, of the organisms originally present in the food together with all those added at various regions of the alimentary canal. The microbic flora of the large intestine is, however, most prominent in the fæces. The total quantity and the proportions of the various kinds of microbes in the fæces varies considerably even in health, depending upon various factors, among which age of the individual and character of the food are very important.

The first meconium passed after birth may contain few or no microorganisms. Within a few hours, however, they appear in the intestinal discharges. The earliest forms are usually large diplococci to which are soon added various bacilli, small diplococci and tetrads. Among the bacilli, a long slender form with a large oval terminal spore, the headlet bacillus of Escherich, is particularly conspicuous. *B. coli* is also present at this time and several gelatin-liquefying forms of bacilli can be isolated in cultures, among them *B.* (*Proteus*) *vulgaris* and *B. subtilis*. Anaerobic cultures demonstrate the presence of *Bact. Welchii*, *B. edematis* and *B. bifidus*.

As the meconium is replaced by the residue of the ingested mother's milk, the previously variegated bacterial flora suddenly becomes very simple and during the whole period of exclusively breast feeding the stools contain enormous numbers of the Gram-positive micro-aerophilic *B. bifidus* of Tissier, with only small numbers of *B. coli* and very few cocci. The dominance of *B. bifidus* may readily be demonstrated by making a series of dilution cultures in tall tubes of glucose agar, according to the method of Veillon, and incubating them for five days or more. When the child begins to take cow's milk there is a sudden increase in the relative numbers of *B. coli* and streptococci and with the addition of starchy foods to the diet the fæcal flora gradually comes to resemble that of the adult.

In the healthy adult taking a mixed diet, the fæcal flora consists for the most part of Gram-negative bacilli of the type of *B. coli*. There are also many diplococci, a few small Gram-positive bacilli (*B. bifidus?*) a small number of *Bact. Welchii* and its free spores, a few representatives of the *B. edematis* group and a variable number of slender spirochetes. Aerobic plate cultures on agar or gelatin often bring to development only *B. coli*. When a vegetarian diet rich in indigestible residue is consumed, the diplococci are much diminished in numbers; numerous large bacilli, *Bact. Welchii* and *B. subtilis*, take their place. The consumption of excessive quantities of meat and starchy foods may lead to a considerable increase in the numbers of the *Bact. Welchii* group and some of the bacteria of this group may be stained brown or blue with iodine because of the granulose which they contain. The bacteria normally present in the fæces are produced almost altogether by multiplication within the intestine. It is nevertheless possible for swallowed organisms to appear alive in the fæces even though incapable of growth within the digestive tube.

The introduction of foreign organisms capable of multiplication in the gastro-intestinal canal may lead to a marked alteration in the quantitative relationships of the fæcal bacteria or even to the disappearance of certain microbic forms previously present. Thus in cholera, the vibrio of this disease may occupy the intestinal canal so completely that the usual fæcal bacteria can no longer be found with the microscope. By feeding acid-resisting lactose-fermenting bacteria, such as *Bact. bulgaricum* along with considerable quantities of milk, it is possible to suppress the putrefactive anaerobes, *B. edematis* group, which prefer a neutral or alkaline medium. The swallowed bacteria are manifestly, therefore, of some importance in determining the character of the fæcal flora, but they are, after all, usually less important in this respect than the chemical composition of the food itself. In every case the original intestinal flora has to be reckoned with as a most essential element.

The daily excretion* of bacteria in the fæces of healthy men, is, on the average, about 33 million million bacterial cells. The washed and dried substance of these bacteria amounts to about 5⅓ g. per day. From one-sixth to one-fifth of the weight of the dry fæces and probably about a third of the moist fæces consists of bacterial substance. The nitrogen carried away by these fæcal bacteria represents a daily loss of 0.5 to 1.0 g.

In addition to the bacteria, one often finds in the fæces yeasts and protozoa. Of the latter *Entamœba coli* is probably an almost constant inhabitant of the intestinal tract and its numbers are often augmented in mild chronic digestive disturbances. The flagellates, *Lamblia intestinalis* and *Trichomonas intestinalis* are found less frequently. A few other protozoa occur in disease.

The physiological effects of the normal intestinal bacteria are not fully understood. Some observers have maintained that continued life and growth would be impossible without the bacteria of the digestive tract, ascribing to them an essential part in the nutrition of the body. The experiments of Cohendy† seem now to have disproven this hypothesis. There can be no doubt that the bacteria do enter intimately into intestinal digestion and in some instances bring about changes beneficial to their host, such as the digestion of cellulose, whereas when furnished other food they may exert a harmful influence, as for example in excessive intestinal putrefaction.

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* MacNeal, Latzer and Kerr, Jour. Infect. Diseases, 1909, VI, 123.

† Cohendy, Annales de l'Institut Pasteur, 1912, XXVI, 106.

In diseased conditions of the gastro-intestinal tract one finds more or less well-marked alterations in the fæcal flora. These changes include quantitative change in the total bacterial output, change in the proportional relationships of the various normal types and finally the appearance of new or foreign types of organisms, either harmless or pathogenic. In many instances there is furthermore a distinct tendency for some members of the normal intestinal flora to assume pathogenic properties and invade tissues rendered less resistant by disease.

Among the intestinal microörganisms which may assume pathogenic rôles at times may be mentioned *B. coli*, *B. vulgaris*, *Ps. pyocyanea*, *B. bifidus*, *Bact. Welchii*, the streptococci, micrococci and *Trichomonas intestinalis*. Among the definitely pathogenic forms are *B. typhosus*, *Msp. comma* (*Sp. choleræ asiaticæ*), *B. paratyphosus*, *B. enteritidis*, *Bact. dysenteriæ*, *Bact. anthracis*, *Bact. pestis*, *Bact. tuberculosis*, *Entamœba dysenteriæ* (*histolytica*), *Coccidium hominis* and *Lamblia intestinalis*.

The technical procedures necessary for the recognition of some of these organisms in the fæces and for their isolation in pure culture are in some instances highly specific. Thus if one is searching for *B. bifidus* it is best to employ dilution cultures in tall tubes of glucose agar inoculated with fæces of a healthy nursling. The same material plated on gelatin will yield only colonies of *B. coli*. *Bact. Welchii* is most readily isolated by pasteurizing a suspension of the fæces and introducing it into blood broth or litmus milk in a Smith fermentation tube. The cholera organism is searched for by introducing considerable quantities of fæces into flasks of pepton-salt solution and transplanting from the surface film after six hours to new flasks. On account of its very rapid multiplication in this medium the cholera germ, if present, outstrips the other fæcal bacteria. Subsequently it is necessary to apply specific agglutination tests to the spirals thus obtained in order to recognize them with certainty. The typhoid bacillus, on the other hand, is sought by inoculating media containing substances which restrain bacterial growth in general without inhibiting the growth of

B. typhosus. Broth and agar containing brilliant green, agar containing fuchsin and sulphite (Endo's medium) and agar colored with eosin and methylene blue are employed for this purpose. The tubercle bacillus when present, may sometimes be separated by digesting the fæces in alkali or in antiformin solution, washing the residue and planting it on Petroff's medium* or injecting it into guinea-pigs. *Entamœba coli* and *Entamœba dysenteriæ* should be searched for with the microscope in fresh warm fæces obtained after a dose of salts.

This brief mention of a few procedures indicates the specialized character of the microbiological technic in this field. The laboratory worker will find it essential to consult the general references below and to study carefully the original papers bearing upon his field of work.

GENERAL METHODS OF STUDY

COLLECTION OF MATERIAL.—Material for microbiological study may be obtained from the mouth, fauces or pharynx by means of a sterile cotton swab, by the ordinary platinum loop or other instrument suitable for the special purpose in view. This material should be examined promptly, or, if this is impossible, it should at once be spread upon slides for subsequent microscopic study and, if cultures are to be made, it should be suspended in sterile salt solution, or better in sterile ascitic fluid, and refrigerated until the proper media can be inoculated. From the stomach, fluid may be readily obtained through a stomach tube and the contents of the duodenum or of upper portions of the small intestine may be withdrawn through the slender duodenal tube of Einhorn. The contents of the lower part of the small intestine and the upper part of the large intestine can be readily obtained only at surgical operations upon the intestine, at autopsies or from individuals in whom an intestinal fistula has been established. The contents of the lower part of the large intestine are best collected by means of a special glass instrument in the case of young children. In older children and adults a natural stool or one obtained after salts or other cathartic may be utilized.

In every instance, contamination of the material with extraneous organisms is to be strictly avoided by careful sterilization of implements and receptacles and any alteration of the specimen after collection must be reduced to the minimum by examining it promptly, although, for some purposes the use of refrigerated specimens may be permitted.

The quantity† of microbic cells present may be ascertained by numerical count of those present in an accurately measured portion of the material, or if they are very abundant they may be physically separated out from a weighed portion by fractional sedimentation in the centrifuge, after which they are dried and weighed (method of Strasburger).

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* Petroff, Journ. Exp. Med., 1915, XXI, 38.

† For detailed directions concerning quantitative methods as applied to the study of fæca bacteria, see MacNeal, Latzer and Kerr. Journ. Infect. Diseases, 1909, VI, 123; *ibid.*, 1909, VI. 571.

A preliminary classification of the recognizably different kinds of microbes should be made by microscopic examination of film preparations stained (1) with Loeffler's methylene blue, (2) by Gram's method, (3) by the Ziehl-Neelsen method and (4) simply with Lugol's solution. It is best to count from 500 to 1,000 microbic cells as they are met with in successive microscopic fields and to classify them according to form, size and structural details brought out by the different stains. Permanent records and, if possible, permanent mounted preparations should be preserved, so that the microbes subsequently brought to development in the cultures may be identified with some of those present in the microscopic picture of the original material.

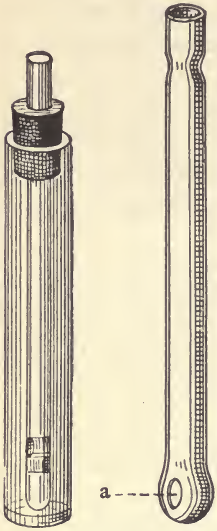

FIG. 155.—Two types of instrument for obtaining fæces from infants for bacteriological examination. (*After Schmidt and Strasburger.*)

Cultures are best made upon a quantitative basis, employing for inoculation measured amounts of accurately prepared dilutions of the original material. There is no single culture medium or method which can be relied upon to give any approximate conception of the numerical relations of the microbes of the digestive tract. Each cultural method necessarily favors certain species present in the mixture and allows others to develop only poorly or not at all. Adequate information concerning the quantitative relationships is obtained only by comparing the results of the culture work with the direct quantitative estimations and by fitting the cultural results into the original microscopic picture. A great variety of culture media and culture methods, aerobic, anaerobic and micro-aerophilic, must be employed in making even an incomplete general survey of the microbes from any portion of the digestive tract. For the detection of certain single species, on the other hand, one may sometimes rely upon a single medium, such as blood-agar for the streptococci of the mouth, Loeffler's serum for diphtheria bacilli in the pharynx or blood-broth in fermentation tube for spores of *B. welchii* in the fæces. Thus the numerous time-consuming procedures may be very much abridged and many of them may well be omitted when one wishes to ascertain merely the presence or absence of a certain single species of microbe.

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DIVISION VI

MICROBIOLOGY OF ALCOHOLIC FERMENTATION AND DERIVED PRODUCTS*

CHAPTER I

WINE

Wine may be defined shortly as the product of the alcoholic fermentation of sound, ripe grapes and the usual cellar treatment.

The classifications of wines are numerous and the varieties innumerable. They may be separated, however, into a few main groups, depending on chemical composition and methods of manufacture. *Dry* wines are those in which practically all the sugar has been removed by fermentation; *sweet* wines, those in which enough sugar remains or is added to be noticeable to the taste; *fortified* wines, those that have received an addition of distilled *wine spirits;* and *sparkling* wines, those highly charged with carbon dioxide, produced by supplementary fermentation in the bottle. Each of these groups includes *white* wines made from the expressed juice of the grape, and *red* wines made from both the juice and skins of red grapes.

Wine in the proper sense is therefore produced exclusively from fresh grapes. Much so-called wine is made in many countries from dried grapes or mixtures of grapes and other fruits with sugary materials of various kinds and various coloring and flavoring substances. Some contain no grapes at all. In most countries, these beverages cannot be sold without some qualifying designation, such as modified, ameliorated, or imitation wine, or piquette, plum wine, gooseberry wine, etc.

GRAPE JUICE AND WINE AS CULTURE MEDIA

Grape juice, known technically as *must*, is a sugary, acid, organic solution very favorable to the growth of yeasts and of many other fungi, but unfavorable to most bacteria. Wine is of a similar composition but contains alcohol instead of sugar and is, therefore, less favorable to the growth of most microörganisms. Both liquids are of highly complex composition. Their character as culture media is indicated by the following table:

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* Prepared by F. T. Bioletti.

COMPOSITION OF MUST AND DRY WINE

	Must	Wine
Specific gravity	1.0600 to 1.1090	0.9850 to 1.0000
Fermentable sugar	12.0 to 25.0 per cent	0 to 0.5 per cent
Alcohol by volume	none	8.0 to 15.0 per cent
Acidity (as tartaric)	0.5 to 1.25 per cent	0.35 to 1.0 per cent
Nitrogenous matters (soluble)	0.2 to 0.4 per cent	variable but small
Tannin	Traces	traces to 0.30 per cent
Dry extract		1.4 to 4.0 per cent
Ash	0.20 to 0.70 per cent	0.13 to 0.50 per cent

Fortified wines (sweet wines are usually fortified) usually contain enough alcohol to make them practically antiseptic to all microörganisms.

THE MICROORGANISMS FOUND ON GRAPES

On the surfaces of grapes, as they are brought to the cellar, may be found any of the bacteria and fungi usually carried by the air and by insects. Many of these are incapable of growing in grape must, and are, therefore, without effect on the wine.

MOLDS.—The spores of the common saprophytic molds, *Penicillium, Dematium, Aspergillus, Mucor*, are always present in abundance, and they find in must excellent conditions for development. *Botrytis cinerea*, a facultative parasite of the leaves and fruit of the vine, is also nearly always present in larger or smaller quantities. All these molds are harmful to the grapes and the wine. Some of them, such as *Penicillium*, may give a disagreeable, moldy taste to the wine, sufficient to spoil its commercial value. Others, such as some *Mucors* and *Aspergilli*, may injure the wine but slightly except by destroying sugar and diminishing the alcohol. *Dematium pullulans* may produce a slimy condition in weak white musts and most of them may injure the brightness and flavor to some extent.

On sound ripe grapes these molds occur in comparatively small numbers and being in the spore or dormant condition they are unable to develop sufficiently to injure the wine under the conditions of proper wine making. On grapes which are injured by diseases, rain or insects, they may be present in sufficient quantities to spoil the grapes before they are gathered. On sound grapes which are gathered and handled carelessly, they may develop sufficiently before fermentation to injure or spoil the wine.

An exception to the generally harmful effect of these molds is *Botrytis cinerea* (*Sclerotinia fuckeliana*) which under certain circumstances may have a beneficial action. When the conditions of temperature and moisture are favorable, this mold will attack the skin of the grape, facilitating evaporation of water from the pulp. This results in a concentration of the juice. The mycelium then penetrates the pulp, consuming both sugar and acid, principally the latter. The net result is an increase in the percentage of sugar and a decrease in that of acid. This, where grapes ripen with difficulty, is an advantage, as no moldy flavor is produced. Two harmful effects, however, follow: the growth of the mold results in the destruction of a certain amount of material, and a consequent loss of quantity, which is, in certain circumstances, more than counterbalanced by an increase in quality (wines of the Rhine, Sauternes); again, an *oxidase* is produced which tends to destroy the color, brightness and flavor of the wine. This can be counteracted by the judicious use of sulphurous acid.

YEASTS.—The true yeasts occur much less abundantly on grapes than the molds. Until the grapes are ripe they are practically absent, as first shown by Pasteur. Later, they gradually increase in number and on very ripe grapes often become abundant. In all cases and at all seasons, however, their numbers are much inferior to those of the molds and pseudo-yeasts. The cause of this seems to be that in the vineyard the common molds find conditions favorable to their development at nearly all seasons of the year, but yeasts only during the vintage season.

Investigations of Hansen, Wortmann and others show that yeasts exist in the soil of the vineyard at all times, but in very varying amounts. For a month or two following the vintage, a particle of soil added to a nutritive solution contains so much yeast that it acts like a leaven. For the next few months, the amount of yeast present decreases until a little before the vintage, when the soil must be carefully examined to find any yeast at all. As soon as the grapes are ripe, however, any rupture of the skin of the fruit will offer a favorable nidus for the development and increase of any yeast cells which reach it. Where these first cells come from has not been determined, but as there are still a few yeast cells in the soil, they may be brought by the wind, or bees and wasps may carry them from other fruits or from their hives and nests.

The increase of the amount of yeast present on the ripe grapes is often very rapid and seems to have (according to Wortmann) a direct relation to the abundance of wasps. These insects, passing from vine to vine, crawling over the bunches to feed on the juice of ruptured berries, soon inoculate all exposed juice and pulp. New yeast cultures are thus produced, and the resulting yeast cells quickly disseminated over the skins and other surfaces visited.

The more unsound or broken grapes present, and the more honeydew or dust adhering to the skin, the larger the amount of yeast will be. The same is true, however, also of molds and other organisms.

In the older wine-making districts, much of the yeast present on the grapes will consist of the true wine yeast, *S. ellipsoideus.* The race or variety of this yeast will differ, however, in different districts. Usually several varieties will be found in each district. The idea prevalent at one time, that each variety of grapes has its own variety of yeast seems to have been disproved, though there seems to be some basis for the idea that grapes differing very much in composition, varying in acidity and tannin contents, may vary also in the kind of yeast present. Several varieties of *S. ellipsoideus* may occur on the same grapes. In new grape-growing districts, where wine has never been made, *S. ellipsoideus* may be completely absent.

Besides the true wine yeast, other yeasts usually occur. The commonest forms are cylindrical cells grouped as *S. pasteurianus.* These forms are particularly abundant in the newer districts where they may take a notable part in the fermentation. Their presence in large numbers is always undesirable and results in inferior wine. Many other yeasts may occur occasionally and are all more or less harmful. Some have been noted as producing sliminess in the wine. Many of these yeasts produce little or no alcohol and will grow only in the presence of oxygen.

Pseudo-yeasts.—Yeast-like organisms producing no endospores always occur on grapes. Their annual life-cycle and distribution are similar to those of the true yeasts, but some of them are much more abundant than the latter. They live at the expense of the food materials of the must and when allowed to develop cause cloudiness and various defects in the wine.

The most important and abundant is the apiculate yeast, S. *apiculatus.* According to Lindner this is a true yeast, producing endospores. The cells of this organism are much smaller than those of S. *ellipsoideus* and very distinct in form. In pure culture these cells show various forms, ranging from ellipsoidal to pear-shaped (apiculate at one end) and lemon-shaped (apiculate at both ends). These forms represent simply stages of development. The apiculations are the first stage in the formation of daughter cells, the ellipsoidal cells, the newly separated daughter cells, which later produce apiculations and new cells in turn.

Many varieties of this yeast occur, as in the case of S. *ellipsoideus.* They are widely distributed in nature, occurring on most fruits, and are particularly abundant on acid fruits such as grapes. Apiculate yeast appears on the partially ripe grapes before the true wine yeast and even on ripe grapes is more abundant than the latter. The rate of multiplication of this yeast is very rapid under favoring conditions and much exceeds that of wine yeast. The first part of the fermentation, especially at the beginning of the vintage and with acid grapes, is, therefore, often almost entirely the work of the apiculate yeast.

The amount of alcohol produced by this yeast is about 4 per cent, varying with the variety from 2 to 6 per cent. When the fermentation has produced this amount of alcohol the activity of the yeast slackens and finally stops, allowing the more resistant ellipsoideus to multiply and finish the destruction of the sugar. The growth of S. *apiculatus,* however, has a deterring effect on that of the true wine yeast so that where much of the former has been present during the first stages of fermentation the latter often fails to eliminate all the sugar during the last stages.

When the apiculate yeast has had a large part in the fermentation, the wines are apt to retain some unfermented sugar and are open to the attacks of disease-producing organisms. Their taste and color are defective, often suggestive of cider, and they are difficult to clarify. This yeast attacks the fixed acids of the must, the amount of which is, therefore, diminished in the wine, while on the other hand the volatile acids are increased.

Many other yeast-like organisms may occur on grapes, but, under ordinary conditions, fail to develop sufficiently in competition with apiculatus to have any appreciable effect on the wine. Most of them are small round cells, classed usually as *Torulæ*. They destroy the sugar but produce little or no alcohol.

A group of similar forms, known collectively as *Mycoderma vini*, occurs constantly on the grapes. These, being strongly aerobic, do not develop in the fermenting vat, but under favoring conditions may be harmful to the fermented wine.

BACTERIA of many kinds occur on grapes as on all surfaces exposed to the air. Most of these are unable to develop in solutions so acid as grape juice or wine. Of the acid-resisting kinds, a number may cause serious defects and even completely destroy the wine. These, the "*disease-producing bacteria*" of wine, are mostly anaerobic and can develop only after the grapes are crushed and the oxygen of the must exhausted by other organisms. Practically all grape must contains some of these bacteria, which, unless the work of the wine maker is properly done, will seriously interfere with the work of the yeast, thus causing injury to the wine. The only bacteria which may injure the grapes before crushing are the aerobic, acetic bacteria, which may develop on injured or carelessly handled grapes sufficiently to interfere with fermentation and seriously impair the quality of the wine.

THE MICROÖRGANISMS FOUND IN WINE

Wine microörganisms may be conveniently divided into two groups: those which grow only in the presence of notable supplies of free oxygen, and those which require, or grow better in, the absence of free oxygen.

AEROBIC ORGANISMS. *Mycodermæ*.—If a normal wine, especially one strong in alcohol, is left with its surface exposed to the air, it will usually, in a few days, be covered with a whitish film, thin and smooth at first but gradually becoming thicker and finally rough and plicate. This is what is known to wine-makers as "*wine flowers*." This film consists of yeast-like cells, somewhat longer and more cylindrical than *S. ellipsoideus*, reproducing by budding and forming large aggregations.

Pure cultures show that there are many varieties of this organism differing in the color and texture of the film, in the cloudiness of the liquid and in the character of the deposit. They are called collectively

Mycoderma vini, though one form which has been found to produce endospores has been called *S. anomalus*.

These organisms are strongly aerobic and can develop only on the surface in full contact with the air. They are a serious enemy to the wine, rendering it insipid and cloudy. They attack the extract, fixed acids, and alcohol, producing at first volatile acids and finally causing complete combustion of the organic matters to carbon dioxide and water, destroying the wine completely.

Acetic Bacteria.—The film formed on wines exposed to the air, especially on those of low alcoholic content, will often differ from that due to *Mycoderma vini*. It will be thinner, smoother and consist of bacteria. These are the vinegar bacteria described on page 637. They grow not only on the wine at the expense of the alcohol, but on crushed grapes and must at the expense of the sugar, producing acetic acid in both cases.

Acetic acid in small amounts is produced by the yeast and is a normal constituent of wine. Unless in excess its effect is not injurious. There may be present from 0.12 g. in 100 c.c. in light white wine to 0.14 g. in a heavy red wine without deterioration of quality. In sweet wines, even a somewhat larger amount may be present without causing injury.

Much larger amounts are injurious in two ways. When the acetic acid is perceptible to the taste, the wine is spoiled. When an abnormal amount of acetic acid is produced before or during fermentation it stops or interferes with the work of the yeast. In such cases, the wine "sticks," that is, fails to eliminate all its sugar and becomes especially open to the attacks of other bacteria.

Wines high in alcohol are less liable to acetic fermentation than weaker wines. Sound wines containing over 14 per cent by volume of alcohol are almost immune, but such wines may be spoiled during fermentation by the growth of acetic bacteria on the exposed floating "cap" of pomace or on the crushed grapes, especially at high temperatures.

ANAEROBIC ORGANISMS (*Facultative and Obligate*).—Some of the worst, most frequent, and most difficult diseases and defects of wine to treat are due to organisms which develop only in the absence of oxygen. These organisms are all bacteria and appear to include a large number of forms, though, owing to difficulties of isolation and culture, the different forms have not been well studied or described.

Slime-forming Bacteria.—Musts and wines become slimy rarely through the action of *Dematium pullulans* (Wortmann) and wild yeast (Meisner) in the presence of oxygen but more frequently through the action of bacteria. In most cases only young wines after fermentation and when contained in closed casks or bottles exhibit this defect. A slimy wine has an oily appearance, pours without splashing and in extreme cases, becomes cloudy and will hang from a glass rod in strings.

In such wines, the microscope reveals large numbers of almost spherical or more or less elongated bacteria in long chains. Some observers have noticed a diplococcus and a sarcina. Kayser and Manceau have recently investigated the subject very thoroughly and described a number of forms which are mostly short rods of from 1μ to 2μ by 0.7μ to 1.2μ. One large form, 3μ to $4\mu \times 1.6\mu$ to 1.7μ was also noted. They all form chains, usually of considerable length. They all produce an abundant slimy sheath and stain easily with carbol-fuchsin and other aniline dyes and are Gram-positive (Fig. 156).

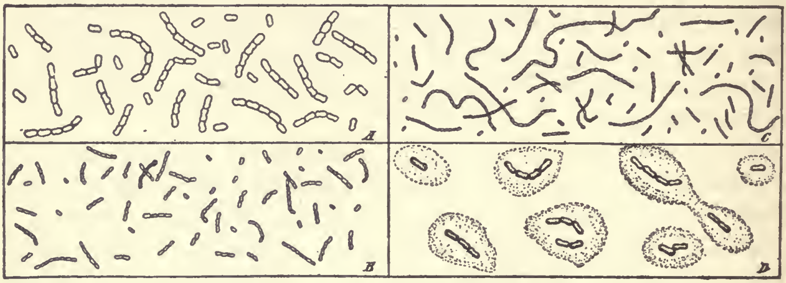

FIG. 156.—Bacteria of slimy wine. *A, B, C,* Pure cultures of various forms; *D,* mucilaginous sheath of slime bacteria. (*After Kayser and Manceau.*)

These bacteria attack the sugar but neither the glycerin nor the alcohol and produce mannit, carbon dioxide, lactic and acetic acids and ethyl alcohol. The disease is usually not serious and disappears under the ordinary cellar treatment. Alcohol above 13 per cent, free tartaric acid, tannin and sulphurous acid in small amounts prevent their growth.

Propionic and Lactic Bacteria.—The most serious and perhaps the commonest disease of wines is characterized by persistent cloudiness, disagreeable odors and flavors, increase of volatile acid and injury to the color or its complete destruction. Wines affected are characterized commonly as *mousey*, *lactic* or *turned* wines (Pousse and Tourne of the French).

The disease is due to bacteria. Enormous numbers are readily revealed by the microscope in badly affected wines. There seem to be several or many closely related forms, all short rod-shaped, isolated in the first stages of the disease, but later forming chains or filaments of various lengths. The most noticeable change caused in the composition of the wine is the decrease of fixed and increase of volatile acidity. The tartaric acid and tartrates are destroyed, and carbonic, acetic, lactic, propionic and other acids formed.

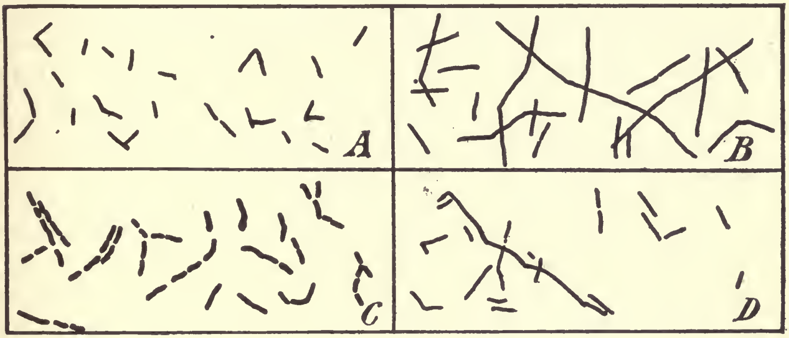

FIG. 157.—Bacteria of wine diseases. *A*, Bacteria of "turned wine," young wine (*After Bioletti*); *B*, bacteria of "turned wine," old wine (*After Bioletti*); *C*, mannitic bacteria (*After Maze and Pacottet*); *D*, bacteria of "bitter wine" (*After Pacottet*).

Light wines of low acidity are most subject to this disease which may be prevented by measures which increase the acidity and alcohol, by rapid and complete defecation and attenuation of the wine with the proper use of sulphurous acid, and finally by timely filtration and pasteurization. Wines noticeably affected can be used only for distilling; those badly affected are valueless.

Mannitic Bacteria.—Very sweet grapes of low acidity in hot climates are subject during fermentation to a similar trouble characterized by increase of volatile acids and a persistent cloudiness and a vapid sweet-sour taste. The disease is commonly confused with the preceding but is caused by bacteria of different forms. The form described by Gayon is a very fine short rod which does not unite in filaments. It attacks the sugar, especially the levulose, producing volatile acids and mannit. The latter may reach over 2 per cent and the former 5 per cent, giving a sweet-sour wine which is completely spoiled.

The bacteria grow abundantly only at high temperatures approaching 40° and can be controlled by cool fermentation, increase of acidity and proper use of sulphurous acid.

Butyric Bacteria.—In the cooler climates, wines, especially old red wines in bottles, often become bitter. This trouble is due to comparatively large rod-shaped bacteria, first described by Pasteur. The cells remain united in angular filaments, short at first, but becoming longer and finally thicker with age by incrustations of coloring matter.

The tannin, coloring matter, and glycerin of the wine are attacked, acetic and butyric acids being formed. In small amounts the bacteria do little or no harm, in larger amounts they may spoil the wine. Means which increase the alcohol, tannin and acidity diminish the liability to the disease. Prompt attenuation and clarification and in extreme cases pasteurization will cure wines not too badly affected.

All the above anaerobic bacteria of wine diseases probably exist in most wines. Which develop most, or whether any develop sufficiently to injure the wine depends on conditions, chiefly the composition of the must and the temperature at which the wine is fermented or stored. Most diseased wines show a mixed infection of several forms. Recently W. V. Cruess has found bacteria in wine containing twenty per cent of alcohol. These bacteria were living and causing cloudiness and increasing the volatile acids.

CONTROL OF THE MICROÖRGANISMS

Given grapes of suitable composition, the quality of the wine depends on the work of microörganisms. The art of the wine-maker consists almost entirely in the control of these microörganisms. His success in facilitating the work of the useful form (true wine yeast) and in preventing or hindering the work of injurious forms determines the quality of his product.

BEFORE FERMENTATION.—On the skins of sound ripe grapes as they hang in the vineyard, the microörganisms are comparatively few and in an inactive condition. When proper methods are used they cannot injure the wine. On broken or injured grapes, the number is greater and the forms more active. If many such grapes occur they should not be mixed with the sound grapes if the best wine is to be made.

Care should be taken to avoid unnecessary bruising of the fruit if it cannot be worked immediately. Molds, wild yeasts and acetic bacteria multiply rapidly on grapes wet with juice.

The sooner the grapes can be crushed and placed in the fermenting vat or pressed the easier it is to obtain a sound fermentation.

Cleanliness is essential. Grapes, which are gathered in moldy, vinegar-sour boxes, hauled in dirty wagons or cars, and passed through dirty crushers, conveyors and presses, may be so completely infected with injurious germs that it is impossible to obtain a good fermentation. The most injurious forms of dirt are must, grapes, or wine, which have been allowed to become moldy or vinegar-sour.

Dust or soil is less injurious and, if excessive, may often be removed by sprinkling, especially is this true if the grapes are too sweet and require dilution.* Washing with antiseptics is not permissible. A weak solution of potassium metabisulphite might be used with benefit if it were not for the difficulty of regulating the amount of sulphurous acid entering the fermenting vessel.

If the grapes have to be kept for some time before crushing, they should be kept as cool as possible to delay the growth of molds. Gathering in the cool of the morning is desirable and if grapes are gathered when warm they should be left in boxes to cool off during the night whenever possible. If the grapes are cool when they reach the fermenting vat, they will neutralize a certain proportion of the heat of fermentation, and the difficulty of avoiding injuriously high temperatures is diminished.

However carefully the grapes are handled, a certain amount of dust containing germs and other injurious matters will reach the vats and presses. In the manufacture of white wines, especially, it is desirable to get rid of these matters before fermentation. This is best accomplished by settling and decantation.

As the juice runs from the press, it is pumped into a settling tank or cask. If it is cold, below 15°, and of full normal acidity, the impurities may settle in twenty to forty-eight hours. If the temperature is higher than 15° and the acidity low, molds and yeasts will develop or fermentation will start and prevent settling. A slight sulphuring with the fumes of burning sulphur or with a solution of potassium metabisulphite is therefore usually necessary. The sulphuring should be as light as possible with acid musts as it tends to preserve the fixed acids. For the same reason it benefits musts of low acidity. In from twelve to twenty-four hours, the must is purged of all its gross impurities including dust, and solid particles derived from the skins and the stems and pulp of the grapes. It may be slightly cloudy or nearly clear. It can then be drawn off into clean casks and fermentation started with yeast. The microörganisms settle only in part but they are all paralyzed temporarily.

This defecation is of great value, ridding the must of substances that would affect the flavor of the wine in the heat of fermentation and eliminating the excess of protein matters that would serve as food for injurious bacteria. Centrifugal machines have been devised to hasten the process of defecation, but their work is imperfect.

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* Formerly a decision of the U. S. Dept. of Agriculture forbade the use of the term "pure wine" when water in even the smallest quantities had been used. By the federal law of 1916 dilution with water up to 35 per cent. is allowed.

Sterilization by heat has been tried for the same purpose but with indifferent success. High heating caramelizes part of the sugar and oxidizes the must, thus injuring the flavor. Discontinuous heating at lower temperatures in an atmosphere of carbon dioxide is preferable but troublesome and expensive. All methods have the defect of extracting undesirable substances from the solid matters which are heated with the must.

Chemical sterilization is still less practicable. No substance could be used for this purpose except sulphur dioxide; this used in sufficient quantities would seriously injure the flavor of the wine. The effect would be totally different from that of the small quantities used in defecation.

All the methods discussed have for their object the diminution or elimination of microörganisms of all kinds. With the injurious forms the true yeast is also removed. The more perfect these methods, the more necessary it is to add wine yeast. Without this addition, in fact, all these precautions may result in harm, for the wine yeast, being present in much smaller numbers than many of the injurious forms, may be completely removed while enough of other forms are left to spoil the wine.

A "starter" of some kind is therefore necessary with defecated must and useful in all other cases.

A Starter.—One method of producing a starter is to gather a suitable quantity of the cleanest and soundest ripe grapes in the vineyard, crush them carefully and allow them to undergo spontaneous fermentation. Perfectly ripe grapes should be selected and the fermentation allowed to proceed until at least 10 per cent of alcohol is produced. If imperfectly ripened grapes are used or the starter used too soon, the principal yeast present will be *S. apiculatus*. Toward the *end* of the fermentation, *S. ellipsoideus* predominates. From 4 to 12 l. (1 to 3 gallons) of this starter should be used for each 400 l. (100 gallons) of grapes or must to be fermented. Too much starter should not be used in hot weather or with warm grapes, otherwise it may be impossible to control the temperature.

This starter is used only for the first vat or cask. Those following are started from the first fermentations, care being taken always to use the must only from a tank at the proper stage of fermentation and to avoid all tanks that show any defect.

An improvement on a natural starter of this kind is a pure culture of tested yeast. Such yeasts are being used extensively in most wine-making regions, usually with excellent results. The methods of use would require too much space to describe here, but they are simple and such as could easily be devised by anyone with some knowledge of microbiological technic. They do not aim at obtaining an absolutely pure fermentation, which is unnecessary, but endeavor to have an overwhelming proportion of a thoroughly tested and suitable yeast which will rapidly and perfectly attenuate the wine before the few injurious microörganisms present have time to do any harm.

During Fermentation.—However carefully the injurious germs have been excluded and the good yeast increased, fermentation will not be successful unless conditions as favorable to the latter and unfavorable to the former as possible are maintained.

The temperature of the crushed grapes or expressed must is of importance. If it is below 15°, unless the weather is warm, the grapes should be warmed to 20° or 25°. Unless this is done, the molds and *S. apiculatus*, which require less heat than *S. ellipsoideus*, will develop more quickly. This is especially true when starters are not used. In the warmer and earlier districts the grapes are practically never too cold. On the other hand, unless there is great carelessness, the grapes are never too warm for the commencement of fermentation. The warmer they are, however, the more artificial cooling will be necessary later, and the sooner it will have to be applied.

For white wine, the crushing must be thorough to facilitate the pressing out of the juice which is fermented alone. For red wine, it is only necessary to break the berries as the fermentation softens the pulp sufficiently for pressing. If the grapes are not crushed, they ferment unevenly and the growth of injurious mold is encouraged.

The must should be thoroughly saturated with air at the beginning of fermentation to insure the multiplication of the yeast. The aeration received in the processes of stemming, crushing and pressing is usually sufficient for this purpose. More aeration would be harmful, injuring the flavor and color of the wine by over-oxidation and promoting the growth of injurious aerobic organisms. An objection to the sterilization of must by heat is the expulsion of the air and the difficulty of replacing it in the proper amount.

The proper use of sulphurous acid in the regulation of fermentation is one of the most important and necessary but least understood parts of the wine-maker's art. Only by this proper use can wholesome wine of the highest quality be produced. Improper use will injure or completely spoil the wine. Its beneficial effects are due primarily to its action on microörganisms, on enzymes and on the color of the wine.

In the small quantities properly used in winemaking, it is antiseptic in a degree varying with the amount. All microörganisms are susceptible to its action in varying degrees. Bacteria are particularly sensitive, molds and psuedo-yeasts less so, while wine yeast is the most resistant of the ordinary forms found in must and wine.

The result of the use of the proper amount of sulphurous acid in crushed grapes and must before fermentation is the almost complete suppression of bacterial action, the discouragement of molds and pseudo-yeasts and the promotion of the growth of wine yeast which is given a clear field unhindered by the deleterious excretions of competitors.

Its action as regards enzymes is hardly less important. It would be impossible to make the finest wines of Sauternes and the Rheingau without its use on account of the oxidase produced by the *Botrytis cinerea* which is abundant and necessary on the best grapes of these regions. In other regions where this mold and others occasionally occur its use is also necessary. In hot climates it is especially useful, not only because bacterial action is more intense in such regions but because of its action in preserving the natural fixed acids of the grape, which are, there, nearly always deficient. This preservation, according to Wortmann, is due to the suppression of acid-consuming bacteria, but experiments of Astruc tend to show that the prevention of the action of unknown acid-destroying enzymes is in part the cause.

Its action on the color of wines is also of importance. By the action of oxygen, the color of red wine is gradually made insoluble and precipitated, and the greenish or golden color of white wine is turned to brown. Both these actions are prevented or much diminished by the use of minute quantities of sulphurous acid.

The most commonly used source of sulphurous acid is the fumes of burning sulphur. Sulphur is burned in a cask and the must caused to take up the fumes by being pumped into the cask through the upper bung hole. It is almost impracticable to apply sulphurous acid from this source to crushed grapes for red wine.

The method is defective in many ways. It is impossible to tell within very wide limits how much sulphur dioxide has been absorbed by the wine. Moreover, the sulphur burns incompletely and the volatilized sulphur acted upon by the yeast may produce sulphuretted hydrogen. Other sulphur compounds are also produced during the burning, to some of which the so-called sulphur taste of wine is said to be due. Several devices have been invented to decrease these defects but none remove them completely; accordingly progressive wine-makers are adopting more reliable sources.

An improvement is the use of potassium metabisulphite ($K_2S_2O_5$) a salt which can be obtained in the requisite purity in commerce containing 50 to 55 per cent by weight of sulphur dioxide. The amount of potash added by this salt in the doses used, is very small, and far within the limits of variation between different wines. By the use of this salt, exact amounts of sulphur dioxide can be applied both to white and red wines. Other sulphites are not permissible.

The best source of the acid, recently brought into limited use, is the liquefied gas, which can be manufactured comparatively cheaply in great purity. By its use all the benefits of sulphurous acid are obtained and the defects eliminated.

Some grapes, owing to their composition, especially their high acidity, are very resistant to the attacks of injurious bacteria. Others, owing to their low acidity or highly nitrogenous nature, are very susceptible. The addition of tartaric or citric acid to the latter has therefore a deterring effect on some of the most dangerous forms. It is seldom necessary, however, to modify the composition for this purpose if the other means of control are used. The addition of acid or its decrease by dilution or neutralization should be solely for the direct improvement of the taste.

The quality and character of the wine depends greatly on the temperature of fermentation. If too low, the fermentation may be unduly prolonged, the wine yeast may have difficulty in overcoming its competitors and the wine may remain inferior and cloudy. With red wine, the desired color, tannin and body may not be secured. On the other hand, if the temperature is too high the results are worse. The growth of bacteria is promoted, injuring the wine by the volatile acid and displeasing flavors produced and preventing the proper action of the yeast. Such wines may remain sweet on account of the failure of the yeast to do its work and become unpleasantly acid owing to the volatile acids produced by the bacteria.

Some means of controlling the temperature is therefore always needed. Where heat is deficient it may be supplied by direct heating of the must or part of it, or by heating the cellar. Where the heat is excessive, it may be diminished by crushing only cold grapes, using small fermenting vats to promote radiation and finally by the use of cooling machines applied directly to the fermenting wine.

The best temperature for fermentation depends on the kind of wine. For light white wines, the maximum should not exceed 25°, for heavier wines 30°, while for heavy red wines where high extract and tannin are required, it may be allowed to reach 35°. Sound wines can be made at all these temperatures.

As already explained, the ordinary processes of treatment of grapes result in sufficient aeration for the multiplication of the yeast. With grapes containing little sugar, this may suffice to complete fermentation. With sweeter grapes, the fermentation usually slackens when the alcohol reaches 11 or 12 per cent by volume or sooner, unless some supplementary aeration is given. With white wine this is seldom done, with the result that the time of fermentation is prolonged. With red wine, the necessary stirring of the pomace to promote color extraction or the pumping over of the must in the cooling process usually gives a large amount of aeration which is sometimes excessive. Too much aeration results in extremely rapid fermentation and consequent difficulty in controlling the temperature. It may also have a deleterious effect on the color, especially if sulphur dioxide has not been used.

In any case, the main part of the fermentation should be over in from three to five days in the case of red and in from seven to fourteen days in the case of white wine. With heavy musts, however, there will still remain from 0.5 to 1 or 2 per cent of sugar. With certain special wines such as Sauternes it is desirable to retain the slight sweetness due to this small amount of unfermented sugar. This is accomplished by the judicious use of sulphurous acid, prompt clarification by filtration or fining and when necessary by pasteurization. The pasteurization tends to remove those proteins which are coagulated by heat and which are the preferred food of bacteria.

In the case of dry wines, protection from bacteria is best obtained by prompt and complete attenuation. Fermentation should not be allowed to cease until all the sugar has disappeared. For this purpose, one, two or more aerations by pumping over are usually necessary immediately after the end of the tumultuous fermentation. The temperature of the wine should not be allowed to fall sufficiently to check the action of the yeast until all the sugar has disappeared.

AFTER FERMENTATION.—As soon as all the sugar has been destroyed in the case of dry wines, or the desired degree of attenuation has been obtained in the case of sweet wines, all the useful work of microörganisms has been accomplished. The quality and safety of the wine then depend on freeing it from all organisms present and preventing the entrance and action of all others.

As soon as bubbles of carbon dioxide cease to be given off, the yeast and other solid matters will settle to the bottom and the liquid become clear. This often occurs before the fermentation is complete. In this case the yeast should be stimulated by aeration as described above.

If the wine is dry, it should be racked (drawn off, decanted) from the sediment into clean casks. The first racking is usually done while the wine is still slightly cloudy during the first month or six weeks to remove the more bulky sediment. If left too long on the yeast the *autophagy* or degeneration of the latter may produce substances which injure the brightness and flavor of the wine.

A second racking is necessary at the end of winter before the spring rise of temperature tends to renew the activity of the microörganisms which always remain in the wine. A well made wine at this time should be perfectly bright and all solid matters consisting of yeast and bacteria, coagulated proteins and crystals of bitartrate should have accumulated in the sediment.

Racking should take place when possible only in settled weather, when the barometric pressure is high. Low atmospheric pressures diminish the solubility of the carbon doxide with which the wine is saturated. Under these conditions, therefore, bubbles of gas are apt to be given off, bringing up particles of sediment and rendering the wine cloudy. However long wine is kept in wooden casks, it will continue to deposit sediment owing to chemical changes due to the action of oxygen which penetrates slowly through the wood. Repeated rackings are therefore necessary, occurring at least twice a year until the wine is bottled or consumed.

Abundant aeration is necessary during fermentation. A moderate supply of oxygen is necessary for the proper aging of wine. Experience has shown that exactly the proper amount of pure filtered air will obtain access to the wine for the latter purpose through the wood of ordinary casks of proper size. If the casks are too small the oxidation may be too rapid, if too large the maturing of the wine may be unduly prolonged. The temperature of the storage cellar is the main modifying factor. The warmer the cellar the larger the casks should be. The range for fine wines is from 50-gallon barrels to 1000 gallon casks. Ordinary wines where aging is unnecessary or impracticable, may be stored in larger containers.

With sound, completely fermented wines, all aeration, other than that due to the porosity of the wood, should be avoided as much as possible. This is accomplished by keeping the casks tightly bunged and completely filled. Evaporation through the wood continually diminishes the volume of wine and the lack must be supplied by *filling up*, at first two or three times a month and later every month or two. The drier the air of the cellar, the more frequent the fillings necessary.

A light sulphuring of the clean casks into which the wine is racked is usual. This should be practised with great caution. Very little is needed with sound wines, especially if it has been used before or during fermentation and a slight excess will injure the flavor. The amount should not exceed 1.25 g. per hectoliter for red or 2 g. for white wine. One-half to one-third of this is sufficient for old wines. The amount can be accurately measured only when using metabisulphite or the liquefied gas. The utility of the sulphur dioxide with perfectly sound wines is to diminish oxidation; with wines liable to disease, to discourage the growth of bacteria.

All manipulation of the wine should be conducted with strict attention to cleanliness. This applies especially to empty casks, pumps and hoses. These should be thoroughly cleaned immediately after use and, if of metal or nonabsorbent material, kept perfectly dry. Utensils of wood, rubber or other porous material should be preserved from bacterial or mold growth with sulphurous acid.

The clarification of a perfectly sound new wine may be facilitated and hastened by thoroughly stirring up the yeast one or two days before racking. The yeast in settling carries down much of the finer suspended matter, thus effecting a rough *fining*. Materials such as kaolin, pure silica sand, charcoal and filter-paper can be used with the same effect. The fining, however, is never perfect and the flavor of the wine is often injured. A very pure clay, known commercially as Spanish clay, is used largely for clearing sweet wines where the flavor is not so delicate. From 75 to 125 mg. per hectoliter are used for this purpose.

The best wines are nearly always fined at least once, immediately before bottling. One or two finings may precede this to hasten aging, defecation and *bottle ripeness*.

The materials used are soluble gelatinous or albuminous substances which are capable of being coagulated and precipitated by some ingredient of the wine. The best of the commonly used substances are isinglass (ichthyocol) 2 or 3 g. per hectoliter, for white wines; the white of fresh eggs, 1 or 2 per hectoliter for red; and gelatin, 10 or 12 g. per hectoliter for either.

The proper quantity of the finings is dissolved in a little water diluted with wine and stirred into the cask. The tannins and acids of the wine cause a gradual coagulation in minute particles throughout the liquid. These particles gradually coalesce, forming larger particles which include all the other floating solid matter of the wine as in a net. These larger particles contracted by the alcohol then settle to the bottom, leaving the wine perfectly bright.

The coagulum consists of a combination of the gelatinous matter and the tannin. Some of the latter, therefore, is removed from the wine. With astringent red wines, this may be an improvement. If there is no excess of tannin present, enough must be added to combine with the finings used. With white wines which contain little or no tannin, this addition is always necessary.

The amount to use varies with the quality of the finings and of the tannin and with the composition and temperature of the wine.

To precipitate commercial gelatin of good quality about an equal quantity of good tannin is necessary; isinglass properly prepared requires only from one-half to one-third this amount. Eggs require only minute quantities.

Specially prepared casein of milk is used for fining white wine. Its chief merit is that the acids of the wine alone cause its complete precipitation and no addition of tannin is needed, though a little is sometimes helpful. Many other albuminous substances such as milk, blood and various proprietary preparations are also used, but they are all inferior to the three mentioned and many of them introduce foreign matters such as milk sugar and bacteria which are a source of danger to the wine.

Wines containing many disease-producing bacteria may be injured by the introduction of finings. The evolution of gases due to the bacterial action may prevent the settling and the protein matters introduced will favor the multiplication of the disease-producing organisms. By the use of 5 to 10 g. of sulphurous acid per hectoliter added to the wine immediately before the addition of the gelatin, the bacteria may be temporarily paralyzed and the finings will then settle and remove the bacteria with the other floating particles.

The bright wine should be racked from the finings very soon after the sediment has settled, especially when disease-producing bacteria are numerous. This will be in from ten to twenty days. If the wine is not clear in three weeks it should be filtered.

Filtering is inferior to fining in producing a perfectly bright wine. It is more rapid, however, and is useful in clearing common wine and wines refractory to fining.

Filters of innumerable forms are used. They are of two main types. For rough clearing of very cloudy wines some form of bag filter is usually employed in which the wine passes through a cloth tissue. The passage at first is rapid and the filtration imperfect. As the solid matter accumulates on the filtering surface, the filtration improves but the passage of the wine is retarded. The first wine is passed a second time through the filter and as soon as the rate of filtration becomes too slow, the operation must be stopped and the filtering surface renewed.

For wines containing little sediment, the filter must be *primed*. This is accomplished by putting some finings in the wine first passed through the filter. The priming is more effective and the output of the filter much increased if a little infusorial earth is used with the gelatin.

For the more perfect clearing of old wines some form of pulp filter is used. These are various devices by which the wine is forced through a mass of cellulose or asbestos pulp and freed from all floating matter. Some of the best of these, carefully used, remove nearly all of the bacteria present.

PROHIBITION AND WINE.—Legislation restricting or prohibiting the manufacture, sale or use of alcoholic beverages has profoundly affected the industry, especially in the United States, where the making of wine and other alcoholic beverages on a commercial scale is now illegal except for sacramental and other specified purposes. Whether it will be permitted as a home industry for family use is still uncertain.

Other uses for wine-grapes are therefore being sought. In the eastern states many can be used for the manufacture of grape juice. In California the most promising means of profitably disposing of the crop are drying and the manufacture of grape syrup. These products, especially the latter, will have some value as foods but their main outlet must be, at first, in those countries where wine-making is permitted.

Wine and vinegar can be made from both of these products by essentially the same methods described for fresh grapes. The dilution of the syrup and the extraction of a must of suitable concentration from the dried grapes offer slight mechanical difficulties which can easily be overcome. The use of starters of selected yeast will be necessary and will offer some difficulty in operation on a small scale.

The quality of the wine will be inferior in some cases and fair to good in others. The color of red grapes is almost entirely destroyed by sun-drying but remains in grapes dried in evaporators. The aromas and flavors of the grapes are modified also more by sun drying and concentration. The effects of these modifications in the resulting wine are both favorable and unfavorable. The wines will have less marked aromas but will age more quickly. The wines made from dried grapes will tend to be high in tannin and extract; those made from grape syrup, low in acid and extract. A combination of the two raw materials will probably give the best results.

CHAPTER II

BEER

Beer is an alcoholic beverage made from certain cereal grains by transformation of the starch to sugar, dilution with water, and fermentation with yeast. There is usually an addition of hops and sometimes of materials containing sugar. The liquid before fermentation is called *wort*.

TYPICAL COMPOSITION OF VARIOUS BEERS

	Lager	Ale	Porter	Weisbier	Temperance beer
Water	90.40	88.30	87.30	92.00	
Alcohol (by vol.)	4.85	8.00	7.00	3.45	2.00
Extract	4.20	5.54	6.45	4.63	3.95
Sugar	1.60	1.33	1.83	1.71	1.98
Lactic acid	0.10	0.20	0.22	0.27	0.04
Ash	0.23	0.30	0.40	0.16	

RAW MATERIALS AND MICROÖRGANISMS OF BREWING

GRAINS EMPLOYED.—Barley, rice and maize are the grains most commonly used, wheat, rye and oats but rarely. Cane and beet sugars and syrups sometimes form part of the fermentable material.

YEASTS OF BEER.—The yeast used is usually one of the many forms of *S. cerevisiæ*. In some spontaneously fermented beer, other yeasts, *Torulæ* and bacteria take part, but in ordinary beers most of these are considered as disease-producing organisms and injurious.

KINDS OF BEER.—The principal varieties of beer are: *lager* beers, fermented with bottom yeasts; *ales*, fermented with top yeasts (and *Torulæ*); *porters*, similar to ale but dark in color owing to the use of caramelized malt; *weisbiers*, in which lactic bacteria are abundant; and certain local types in which bacteria produce considerable quantities of lactic and acetic acids. Many attempts have been made to produce a beverage similar to beer but lacking the alcohol. The "temperance beers" are of this character. Some of them are made by restricting the fermentation or by omitting it. These are little more than wort or decoctions of malt and lack the products of fermentation to which much of the flavor of beer is due. In others the beer is made in the usual way and then by various devices freed from alcohol. The latter are more expensive to produce but are considered of superior quality.

Process of Brewing

Outline.—The manufacture of beer takes place in four main stages. First, a portion or all of the grain is soaked in water, allowed to germinate and then dried. This produces the *malt* which contains the enzymes necessary for the conversion of the starch into sugar and the disintegration of the tissues of the grain. The malt is then crushed (and usually mixed with unmalted cereals or sugar) and heated with water. This constitutes *mashing*. During this process, the starch changes to maltose and dextrins which with other matters dissolve in the water; then bacteria produce a small amount of lactic acid. The resulting solution constitutes the *wort*.

The *wort*, by the addition of yeast is fermented and changed to *beer*. The fourth stage includes all manipulation of the fermented beer to prepare it for consumption.

Malting: Production of Enzymes.—The best malt is made from barley, but for special beers may be made from wheat or other grains. *Steeping* consists in soaking in water to start germination. This requires from thirty-six to seventy-two hours and causes an increase in weight of about 45 per cent. The temperature should be about 12.5°. If higher, injurious molds will develop. If much lower, germination will be retarded. The water should contain little organic matter or chlorides, nitrates or iron salts. A little calcium sulphate is favorable. If it contains many microörganisms it should be sterilized by boiling. A very little sulphite of lime or of potassium may be used to discourage molds.

During *germination* several enzymes appear, of which the most important to the brewer are *diastase* which changes insoluble starch into soluble sugar, rendering it available for the growth of the young plant; *peptase*, which performs a similar function as regards nitrogenous matters; and *cytase* which helps in the disintegration of the cellulose. All these are necessary to prepare for the work of the yeast. When the plumule has grown to about two-thirds the length of the grain, sufficient enzymes have been formed. This requires from about sixteen to twenty days.

The growth of the sprouting seed is at this point stopped by careful drying with artificial heat in a kiln. The *kilning* must be sufficiently rapid to kill the germinating seedling quickly, but not too rapid or at too high a temperature, otherwise the enzymes will be weakened or destroyed. The enzymes are more sensitive when moist, consequently the heat may be increased as drying proceeds. The process commencing at a temperature between 30° and 35° is increased gradually to 50° and 55°. In twelve to twenty-four hours, the malt should appear dry. The temperature is again raised gradually for another twelve to twenty-four hours to 80°–100°. The lower the temperature the lighter the color of the malt. Higher temperatures, especially while the malt is moist, produce dark malt.

As soon as the kilning is finished the *radicles* are *removed* by friction and screening in special machines.

WORK OF ENZYMES AND BACTERIA.—The malt is first *crushed* by pressing between rollers to facilitate the work of the enzymes and the solvent action of the water. If *unmalted grain* is to be used as well, this must be ground and the starch made soluble by heating under pressure with three or four times its weight of water and a little malt to 80°–85° for about an hour.

The methods of *mashing* are very various. They consist in general of mixing the ground malt with warm water, bringing the mass to a temperature of 35° to 45° which is gradually raised to 60°–65° by the addition of hotter water. When the action of the enzymes commences, the heated decoction of unmalted grains is added in various ways, and the temperature controlled by additions of hot water or by heating a portion of the mash. The whole mashing process requires from two to five hours according to the methods used.

During the mashing, the starch is transformed partly into maltose and partly into dextrins. The ratio of these products will vary according to the amount of diastase present and especially according to the temperature used. At about 60° the maximum amount of maltose is produced; at higher temperature (65° to 75°) the unfermentable dextrins increase. The amount of alcohol and the amount of extract in the beer therefore depend to a great extent on the method of mashing.

During the first part of the mashing, while the temperature is about 45°, lactic bacteria develop. If their action is too intense they will render the beer unpleasantly acid. If moderate, the acidity they communicate to the wort is useful in preventing the growth of the harmful butyric bacteria which might develop.

After mashing, the wort is separated from the solid matters by drawing off, extracting the mash with hot water (*sparging*), and filtration. It is then boiled from one to eight hours according to the result desired.

Boiling sterilizes the wort, kills all bacteria and destroys any enzymes which remain. These results are obtained almost instantaneously owing to the lactic acid present. Coagulation of protein substances is also brought about, effecting a clarification of the wort. This requires one or more hours, according to the nature of the wort. It is necessary also in some cases to concentrate the wort, which is done by prolonged heating in open kettles. This may require several hours.

The *Hopping* of the wort takes place during the boiling. Sometimes the hops are added just at the end of boiling; sometimes in two or three portions, one of which may be at the beginning and one after boiling. Hops contain an aromatic essential oil, resins and tannin. The essential oil is quickly soluble and volatile. To preserve its aroma in the beer, the hops must not be boiled too long. The resins are antiseptic and help to preserve the beer. They dissolve with more difficulty and require longer boiling.

FERMENTATION: WORK OF YEAST.—After boiling, the wort is separated from the hop débris by straining. It is then cooled by means of refrigerators consisting usually of serpentine tubes through which cold brine or water runs. The hot wort runs or drips over the outside of these tubes in contact with the air. The final temperature of the wort is from 12° to 18° in top fermentation and 4° to 6° in bottom.

By this means the wort is thoroughly aerated, which is necessary for the proper work of the yeast. It also effects a partial clarification by oxidation which causes a precipitation of solid matters.

The fermentation takes place in two stages, the violent or tumultuous fermentation in vats and the secondary or after fermentation in casks.

During the violent fermentation, the temperature is allowed to reach a maximum of 7° to 9° with light beers, 8.5° to 10.5° with dark and 12° to 20° in top fermentations. At the end of the first fermentation, the beer is cooled gradually to 3.5° or 5.0° and drawn into fermenting casks where the after-fermentation takes place.

The yeasts used in brewing vary very much. Besides the division into top and bottom yeasts, various types of each are recognized. One of the chief characteristics used for this division is expressed by the percentage of the total extract fermented by the yeast. The *Saaz* type leaves all the dextrins and some of the maltose untouched and produces beers light in alcohol and high in extract. The *Logos* type destroys all the maltose and much of the dextrins. The result is high alcohol and low extract. The *Frohberg* type is intermediate. These differences are probably due to differences in the amount and perhaps in the kinds of enzymes.

The yeasts of spontaneously fermenting beers are of various species, *S. ellipsoideus*, *S. pasteurianus* and others.

To produce fermentation, yeast is taken from previous vats so long as the yeast remains sufficiently uncontaminated with foreign organisms. The condition of the yeast is determined by the character of the fermentation, the degree of attenuation, and by microscopic examination. In breweries where modern pure culture methods are not used, the yeast present is always of several forms or types.

In any case, after a certain number of transfers, the yeast deteriorates and finally may become thoroughly infected with bacteria. The bacteria are revealed by microscopic examination. Where pure cultures are used, contamination with foreign yeast is shown by a change in the time of spore formation. By this method a contamination of 1:200 may be discovered.

When the yeast becomes contaminated, a new start must be made with yeast from another brewery, which is uncertain or by a starter of pure yeast, which is the only reliable method.

The new start with pure yeast may be made by employing a kilogram of pure pressed yeast or a corresponding amount of liquid yeast and gradually increasing it to the desired amount by repeated small additions of sterile wort. This must be done with special precautions against contamination. Many large breweries use large pure yeast machines which produce directly sufficient yeast to start a fermenting vat.

After Treatment.—The violent fermentation requires from eight to eighteen days according to the temperature. It takes place in open vats or sometimes, in top fermentation, in barrels. When sufficiently attenuated, the beer is drawn off into large casks where the slow secondary fermentation takes place at a low temperature and the beer clears by depositing yeast and other sediment. The time required for the secondary fermentation is from six to ten weeks or, with certain types of beer, from two to four months or longer.

A certain amount of dissolved carbonic acid is necessary for the quality and keeping of the beer. This is obtained by tightly bunging the casks at a suitable stage of the secondary fermentation.

The clarification of the beer is sometimes assisted by placing a quantity of chips of beech or other tasteless wood in the casks. Top fermentation beers are often fined by the use of isinglass or animal gelatin. Low fermentation beers are usually filtered.

The beer is then ready for delivery to the consumer and is placed in barrels with precautions to retain the dissolved carbonic acid.

The clear beer may be put directly into bottles with the same precautions. Bottled beers which are to be kept for some time or which are to be shipped to a distance are pasteurized after bottling at 60° to 65°.

DISEASES OF BEER

Beer may show defects due to imperfections in the raw material or in the methods of manufacture. These are principally abnormal flavors and lack of clearness.

The diseases properly so called are due to wild yeasts or to bacteria. The disease-producing yeasts may be derived from the starter, from the vessels with which the beer comes in contact, or from the air. They develop most commonly during the secondary fermentation or in the bottle. Some may produce a disagreeable bitterness (*S. pasteurianus* I) or other unpleasant flavor (*S. fœtidus*); many produce a persistent cloudiness (*S. ellipsoideus, S. apiculatus, S. exiguus, S. anomalus*). They are to be combated by preventing contamination, by proper attenuation and by pasteurizing.

Bacterial diseases were more common before effective methods of purifying yeasts were known.

Many forms of lactic bacteria may affect the beer, rendering it acid and cloudy. They occur principally where the temperature is allowed to become too high and where proper care in the cleaning and sterilization of utensils is not exercised.

Acetic bacteria may occur under the same conditions and give a taste of vinegar to the beer. They are more common in top fermented beers.

Various forms of *Sarcinæ* may cause persistent cloudiness, acid, unpleasant flavors or both. This contamination may be from the air or the water and is relatively common. Their growth is most rapid at 16° to 20° and is retarded by the antiseptic properties of hops.

Several kinds of bacteria, bacilli, cocci and sarcinæ may cause the beer to become slimy or viscid and injure the flavor. This trouble is particularly common in spontaneously fermented beer.

Wort and beer, being organic solutions containing very little acidity, are favorable media for the growth of bacteria, many forms of which may cause trouble. With modern methods of using pure yeast, cleanliness and the pasteurization of bottled beer, diseases can be controlled.

CHAPTER III

MISCELLANEOUS ALCOHOLIC BEVERAGES AND PRODUCTS

CIDER AND PERRY

These beverages are made by the alcoholic fermentation of the juices of apples and pears respectively and come next to wine and beer in the quantities produced.

The composition of the fruit varies very much according to the variety, especially in the matters of acidity, tannin and pectic substances. The following analysis is that of a good cider apple:

Sugar	167.0 g. per liter.
Tannin	2.4 g. per liter.
Acidity (as sulphuric)	1.6 g. per liter.

The pectic matters vary from 2 g. to 25 g. per liter but should not be too high. Pears contain usually about the same amount of sugar as apples, more tannin and much less pectic substances.

The microörganisms occurring naturally on the surface of the fruit are similar to those occurring on grapes, but special forms of *Saccharomyces* are found. Pure cultures of wine yeast are used successfully in cider making where a perfectly dry cider is wanted. Where a small remnant of unfermented sugar is desired, the difficulties of using pure cultures have not yet been overcome. The wild yeasts occurring on the fruit in large quantities usually take precedence.

Attempts to sterilize the juice by heating have not been successful owing to the production of a persistent cloudiness. Sulphurous acid is even more effective than in grape juice in delaying or preventing the action of the microörganisms. Its use must therefore always be supplemented by a starter of pure yeast.

The principles of the control of the microörganisms, good and bad, are the same as in wine making. The same care in gathering and keeping the fruit and in extracting and handling the juice are necessary.

The fermentation is similar to that of wine, but the cider should be taken off the yeast sooner in order to promote clarification and the retention of a little unfermented sugar.

Cider is subject to the same bacterial alterations as wine and requires the same treatment. It is more difficult to keep when made in the ordinary way and is usually consumed during the first year. It is particularly subject to turning brown, owing to the large amount of oxidase present in apple juice.

The use of sulphurous acid for preliminary defecation, pure yeast in the fermentation, and fining, followed by pasteurization soon after the fermentation, seem to offer the best means of improving present methods.

These methods were introduced into a cider-vinegar factory in California by W. V. Cruess with excellent results.

FERMENTED BEVERAGES OF VARIOUS FRUITS

Many other fruits, especially those rich in sugar and with moderate acidity, are used locally to produce alcoholic beverages. The methods of fermentation are similar to those used in wine making, but additions of sugar and water are usually made to correct defects of composition. Very often distilled alcohol is also added after fermentation to preserve the liquid, which is thus rendered unsuitable for an ordinary beverage.

HYDROMEL OR MEAD

An alcoholic beverage made by the fermentation of honey and water is much used in eastern Europe.

Honey contains from 65 to 74 per cent of reducing sugars and from 2 to 10 per cent of saccharose. It is diluted with water to reduce its concentration to 22° Bal.*–24° Bal. A few yeast cells are usually present in the honey but these are of various kinds and often unsuitable. The use of a good pure yeast is therefore advisable. As honey contains little mineral or nitrogenous yeast food, an addition of nutritive substances is often necessary.

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* "Balling" refers to the degrees of the special hydrometer for determining the specific gravity of saccharine solutions such as must or beer wort. Its purpose is to indicate directly the percentage of solids in solution at a temperature of 60°F.

The following formulæ are recommended by Kayser and Boullanger to be used in one liter:

A.	Dicalcic phosphate	1 g.
	Ammonia	2 g.
	Bitartrate of potash	2 g.
	Magnesium sulphate	0.1 g.
B.	Maltopeptone	1.5 g.
	Bitartrate of potash	1.5 g.
	Ammonium phosphate	1.0 g.

The same results may be obtained by mixing from 20 to 50 per cent. of the juice of grapes, apples, or other acid fruits with the diluted honey.

MISCELLANEOUS FERMENTED BEVERAGES

Fermented beverages of some kind are made in practically every part of the world. They are very numerous and varied but fall naturally into three groups; those made from the sweet juices of fruits or other plants in which the methods of manufacture resemble those of wine making; those made from starchy materials in which the methods resemble those of brewing; and finally those made from the milk of cows or other mammals which are discussed in Chapter IV, Div. IV.

Belonging to the first group are numerous beverages made from the juices of sugar cane, various palms, and tropical fruits. The best known of these is the MEXICAN PULQUE made by the spontaneous fermentation of the sweet juice of the agave. Little is known about the microflora concerned, but it includes alcohol-forming organisms which produce about 6 per cent of alcohol, and bacteria which cause rapid deterioration and spoiling of the fermented product. The pulque is ready for consumption twenty-four hours after the commencement of fermentation and cannot be kept more than a day or two.

Of the beverages produced from starchy materials the Japanese SAKE, RICE BEER, has been most studied. It is made from rice by the diastatic action of *Aspergillus oryzæ* and yeast fermentation. The process includes three stages. First the preparation of *koji* which consists of steamed rice on which the spores of the fungus are sown and allowed to grow at 20° until the whole mass is penetrated with mycelium. The next stage is the preparation of *moto* which is a thick liquid consisting of steamed rice, water and *koji* in which the fungus transforms the starch into sugar at 0° to 10° in a few days. Fermentation then starts spontaneously, alcohol being produced by the action of several yeasts and lactic acid by bacteria, both present accidentally. In about two weeks the moto is ready. The last stage is the principal fermentation which occurs on mixing together steamed rice, koji, moto and water. This requires two weeks. The liquid is then separated, cleared and stored. It contains a considerable amount of alcohol and can be kept and aged like wine. Sake is said to average 18 per cent of alcohol and may reach 24 per cent, the highest alcohol content known to be produced by fermentation.

POMBE is a kind of beer made in Africa from millet seed by sprouting to saccharify the starch and subsequent spontaneous fermentation in water. It is interesting as the source of the genus *Schizosaccharomyces* which appears to take the main part in the fermentation.

GINGER BEER is an acid, slightly alcoholic beverage made by the fermentation of a 10 to 20 per cent solution of sugar containing a few pieces of ginger root. The fermentation is induced by adding small pieces of the so-called ginger-beer plant which consists of *Bact. vermiforme* and *S. pyriformis*. The bacteria form a thick gelatinous sheath and seem to live symbiotically with the yeast, each developing best in the presence of the other.

DISTILLED ALCOHOL

INTRODUCTION

USES AND SOURCES OF ALCOHOL.—Distilled alcohol is used as a beverage and a medicine or for innumerable purposes in the arts and industries. Certain methods and sources employed for the latter purposes are inadmissible for the former.

In all cases, it is made by the preparation from saccharine or starchy substances of a sugar solution suitable for the work of yeast, the fermentation of this solution, and, finally, the distillation of the alcoholic liquid.

Where the raw materials are sugary, methods similar to those of wine-making, and where starchy, to those of brewing, are employed, modified to suit the conditions of each case.

The principal potable alcohols are *brandy*, made from grapes, *rum* from sugar cane, and *whiskey* from rye or other grains. Many other sources are used and any fermented beverage will, by distillation produce a potable spirit varying in character and quality with the source. Industrial alcohol may be made from any substance capable of undergoing alcoholic fermentation, the limiting factor in practice being, principally, the cost of the raw material per unit of alcohol.

METHODS

PREPARATION OF THE SUGAR SOLUTION.—*Saccharine Raw Materials.* When spirits are to be made from grapes or other fruit, the juice is fermented in the same way as for the corresponding beverage and then distilled. The juice, however, is diluted to 20° Bal. or less, as it is not necessary or desirable to have too much alcohol in the fermented liquid. The product is consumed directly as brandy or used to fortify sweet wines. The principal fruits used besides grapes are apples, peaches, plums and cherries.

Industrial alcohol has been made from inferior or spoiled fruits and from cannery wastes, but the cost per unit of alcohol is usually high. The difficulties of fermentation are great, owing to the presence of large quantities of molds and other injurious organisms, and the extraction of the juice is troublesome. A careful use of sulphites and pure yeast much simplifies the process.

Sugar cane and its products are used in several ways to produce alcohol. To a limited extent the juice of the cane is fermented directly and distilled. The product is known as *Jamaica rum.* Much larger quantities of alcohol are manufactured from the cane-sugar molasses and appear in commerce as *rum, taffia, arrack* or *neutral spirits.*

For the making of Jamaica rum the juice is pressed from the crushed canes, and diluted with 20 per cent of vinasses (the residue of a previous distillation) to increase the acidity, and give the required flavor.

Cane molasses which contain from 50 to 60 per cent of fermentable sugar are diluted with water or vinasses to 15°–18°Bal. and partially neutralized with lime when the acidity is excessive.

One of the principal sources of industrial alcohol is the *sugar beet.* This alcohol is also used for the adulteration or imitation of potable spirits.

It may be made by the direct fermentation of the beet juice, extracted by grinding and pressing, by methodical maceration or by diffusion. Sulphuric acid is added during extraction. This facilitates the extraction by setting free organic acids, and represses the growth of injurious microörganisms. The amount used should be such that a minute quantity of sulphuric acid remains free.

Most beet alcohol is made from the coarser *molasses* of the sugar factories. The molasses are diluted to 20°–30° Bal. with water, further diluted and heated with steam and acidified with sulphuric acid. The sulphuric acid neutralizes the lime which has been used in the manufacture of the sugar, sets free the volatile acids and breaks up the nitrites producing nitrogen peroxide. The liquid is then boiled for about one quarter of an hour to drive off the volatile acids and the oxides of nitrogen which would prevent yeast fermentation. The liquid after cooling is then fermented with yeast.

Starchy Raw Materials.—In the preparation of a fermentable solution from starchy materials three methods for the conversion of the starch into sugar may be used, depending respectively on the action of malt, dilute mineral acids, and certain molds.

The malt used in saccharification may be made, in a manner similar to that described for brewing, from barley, oats, rye or maize. As the object in this case is to cause complete conversion of the starch with as little malt as possible, the malt should have the maximum diastatic power. For this reason, germination should be carried further than for brewing and the malt used green. Drying the malt destroys half its diastase.

The conversion may also be accomplished by boiling one part of grain in four parts of water with hydrochloric or sulphuric acid. With the former acid, 10 per cent of the weight of the grain is used and 5 per cent with the latter. The conversion requires from eight to twelve hours' boiling. The starch is first converted into dextrins and then into glucose. If the boiling is too prolonged some of the glucose may be lost by conversion into caramel. The amount of acid and the time of boiling may be much reduced by operating under 2 to 3 kg. pressure. In this case 200 liters of water are heated with 100 kg. of grain and 4 kg. of acid. Conversion occurs in from 40 to 60 minutes. The acidity is reduced with calcium oxide or calcium carbonate before fermentation.

The power of certain molds, especially mucors, to convert starch into sugar has been utilized. *Mucor rouxii* found in Chinese yeast, *Mucor oryzæ* in Ragi, and related forms have been used for this purpose. This is known as the *Amylo Process.* The grain is first soaked for a few hours, then heated with twice its weight of water under a pressure of three and a half to four atmospheres until soft and the starch rendered soluble. The liquefaction of the starch is facilitated by slightly acidulating the water with hydrochloric acid. The mixture is then cooled to 38° and inoculated with a pure culture of the *Mucor.* A current of filtered air is then passed through the mass for twenty-four hours, by which time the mycelium has permeated the mass. The temperature is then reduced to 33°, pure yeast added and aeration continued for twenty-four hours longer to promote the multiplication of the yeast. Conversion of the starch and fermentation of the sugar then continue together. The mucor is capable of fermenting the sugar and producing alcohol, but the yeast acts more rapidly.

The malting process is the most commonly employed. The acid process destroys a greater part of the value of the residues of distillation and the amylo process, requiring costly special equipment and large expenditures for fuel, has not come into general use.

The starchy substances used being usually neutral or of low acidity the sugar solutions produced would be very liable to bacterial invasion unless means of prevention were used.

In the amylo process, the sterilization of the solutions and the use of pure cultures accomplish this end. In the acid process, the minute quantity of free mineral acid remaining in the completed solution prevents any considerable growth of bacteria. In the malting process the injurious bacteria are restrained by lactic acid produced by lactic bacteria, originating in the malt or in the yeast starter. The requisite. bacteria are obtained by keeping the starter or mother yeast at 50° to 58° for a certain time. This is a favorable temperature for lactic and too high for the development of acetic or other injurious bacteria. When the acidity of the solution reaches 3.5 g. to 5 g. per liter the dangerous butyric bacteria cannot develop.

Pure lactic acid may be added immediately after saccharification and the loss of sugar due to the action of the lactic bacteria avoided, but the high cost of the pure acid prevents the practice.

Yeast being much less sensitive to the presence of certain antiseptics than bacteria it is possible to control the latter by the addition of suitable amounts of an antiseptic to the sugar solution. In certain cases moreover by gradually increasing the amount, yeast can be accustomed to concentrations of antiseptics which render the growth of bacteria impossible. In Effront's method for the preparation of distillation material, hydrofluoric acid is used. This acid is added to the mother yeast at the rate of 10 g. per hectoliter and to the sugar solution in somewhat smaller amounts. This results in the inhibition of lactic, butyric and other bacteria and an increase in the fermentative power of the trained yeast.

FERMENTATION.—The sugar solution properly diluted and acetified or sterilized is fermented by the addition of a mother yeast, usually taken from a previous fermentation.

The original yeast may be obtained by a spontaneous fermentation as is usual in the manufacture of rum. Such a yeast is always impure, containing various yeasts, molds and bacteria, and is therefore very variable and uncertain in its results.

In the fermentation of beet juice and beet molasses, beer yeast of the Frohberg type or special distillers yeasts are used. A starter or mother yeast is prepared for each vat or the process is made continuous by leaving one-third to one-half of the contents of a fermented vat to start a fresh addition of the sugar solution. With the latter method the yeast in time becomes weak and badly contaminated and a new start must be made with fresh yeast.

In the fermentation of solutions made from potatoes, corn or other starchy substances, each vat is started with a mother yeast. The temperature should be kept below 30° by means of refrigeration, otherwise alcohol will be lost by the multiplication of bacteria.

By the use of pure yeast, the yield in alcohol is greater as no sugar is wasted in the production of lactic acid. The cost, however, is greater owing to the necessity of the use of more heat in sterilization.

The fermentation of sugar-cane molasses for the production of arrack is brought about by the use of a mother yeast called *tapej*, prepared from *ragi* or Java yeast.

Tapej is made by mixing powdered ragi with boiled rice. In two days the rice is reduced to a semi-fluid condition and contains bacteria, molds and yeasts. The bacteria seem to have no part in the process but when too numerous are injurious. The mold *Mucor oryzæ* converts the rice starch into sugar and the yeast *S. vordemanni* produces alcohol from the sugar. The other molds present are more or less injurious.

CHAPTER IV

THE MANUFACTURE OF VINEGAR

ACETIC FERMENTATION

NATURE AND ORIGIN OF VINEGAR.—Vinegar is a condiment made from various sugary or starchy matters by alcoholic and subsequent acetic fermentation. It should contain from 4 to 8 per cent of acetic acid and natural flavoring, coloring and other matters, varying according to its origin.

Acetic acid (CH_3COOH) is a monobasic organic acid, the second in the fatty acid series. It is a colorless liquid with a strong suffocating odor, crystalizing when pure at 16.7° and at lower temperatures when diluted with from 1 to 13 per cent of water. Its specific gravity is 1.08 at 0° and it boils at 118° under 760 mm. pressure, producing an inflammable vapor. It is a solvent of many organic substances and is soluble in water and alcohol in all proportions.

The metallic acetates are poisonous and are formed in most cases by simple contact of metal and acid. Certain alloys of tin resist the action of the acid.

Acetic acid is formed by the oxidation of ethyl alcohol which takes place in two stages according to the following reactions:

$$\underset{\substack{\text{Ethyl}\\\text{alcohol}}}{2C_2H_5OH} \underset{+}{+} \underset{\text{Oxygen}}{O_2} \underset{=}{=} \underset{\substack{\text{Acetic}\\\text{aldehyde}}}{2CH_3CHO} \underset{+}{+} \underset{\text{Water.}}{2H_2O}$$

$$\underset{\substack{\text{Acetic}\\\text{aldehyde}}}{2CH_3CHO} \underset{+}{+} \underset{\text{Oxygen}}{O_2} \underset{=}{=} \underset{\text{Acetic acid.}}{2CH_3COOH}$$

These reactions may be brought about by chemical means, but in practice they are due to the action of certain microörganisms, mainly bacteria. Acetic acid is also made by the distillation of wood but the product is not suitable for consumption.

VINEGAR BACTERIA.—If wine, beer or a similar organic solution containing alcohol, is exposed freely to the air it soon becomes covered with a film, the alcohol disappears, is replaced by acetic acid and the liquid is converted into vinegar.

This film, the *Mycoderma aceti* of Pasteur, consists of bacteria cohering by means of a glutinous sheath surrounding each cell, forming a zoöglea. If the film is undisturbed, the liquid remains clear until converted into vinegar, if disturbed, portions may sink, new films form and finally a large gelatinous zoögleic mass, "the mother of vinegar," may form in the liquid.

Sometimes, especially on liquids containing sugar and large amounts of alcohol, such as sweet wines, the film formed consists, not of bacteria, but of a yeast-like fungus, *Mycoderma vini*.

Wines which have been sterilized, often remain without acetifying for a considerable time. Those containing free sulphurous acid acetify slowly and with difficulty. Ordinarily at warm temperatures, exposed wines develop a bacterial film very rapidly owing to the almost constant presence of some acetic bacteria in all wines.

Hansen was the first to show that the vinegar bacteria included more than one species. He isolated and described three species concerned with the spontaneous souring of beers. Later it was shown by A. J. Brown, Henneberg, and others that several other species commonly occurred in vinegar factories and that many more were capable of producing acetic acid in small amounts. The species which have been most thoroughly studied and which seem to occur most usually in vinegar factories are *Bact. aceti*, *Bact. pasteurianum*, *Bact. kützingianum*, *Bact. xylinum*, short descriptions of which follow:

Bacterium aceti (Kützing), Hansen. This species consists of rods about 1μ or 2μ in length, somewhat constricted in the middle and lying in parallel chains in the surface film. This film is moist, smooth, veined, and forms in about twenty-four hours at 34°. On wort gelatin, it forms gray, waxy, raised colonies which are usually round, with unbroken edges but sometimes star-shaped and consisting of separate rod-shaped cells.

Bacterium pasteurianum, Hansen.—The cells of this species are somewhat larger than those of *aceti* and more commonly produce thread-like and swollen involution forms. The film is dry and soon becomes wrinkled. Colonies on wort gelatin are smaller than those of *aceti*, rugose, and the cells retain their arrangement in chains. The mucilaginous sheath is stained blue with iodine-potassium iodide solution (saturated solution of KI colored brown by the addition of a few drops of an alcoholic solution of I), in this differing from *Bact. aceti* (Fig. 158).

Bacterium kützingianum, Hansen.—The cells resemble those of *Bact. aceti*, but are usually free or in pairs. The film resembles that of *Bact. aceti* but has a tendency to climb up the sides of the flask above the liquid. The colonies on wort gelatin are smooth and shiny. The mucilage stains blue with the iodine solution.

Bacterium xylinum, A. J. Brown.—This species forms a thick tough, leathery film, the gelatinous substance of which stains blue with iodine and sulphuric acid.

B. acetigenus, *B. oxydans*, and *B. industrius* are motile species.

All species are strictly aerobic and grow quickly only when freely supplied with oxygen. This oxygen is necessary for the acetification of the alcohol. Duclaux has calculated that one centigram of the bacterial film is capable of uniting 1.3 g. of oxygen to alcohol, 130 times its own weight. The optimum temperature for most species is about 34° and the range of temperature at which they grow is between 4° and 7° to 42°. They all form acetic acid from ethyl alcohol, propionic acid from propyl alcohol and most of them gluconic acid from dextrose. *B. industrius* and *B. oxydans*, according to Henneberg, can form acids from a large number of sugars and related substances, including saccharose, maltose, starch, dextrin, glycerin and mannit.

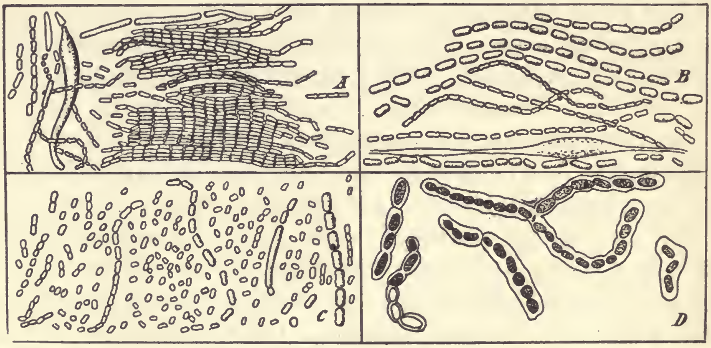

FIG. 158.—Vinegar bacteria. *A*, *Bact. aceti; B*, *Bact. pasteurianum; C*, *Bact. kützingianum; D*, *Bact. pasteurianum*, showing mucilaginous sheath. (*After Hansen.*)

The presence of too much alcohol prevents the growth of acetic bacteria, the limit being about 14 per cent under manufacturing conditions. At 14 per cent and above, the film forms with difficulty, and the oxidation of the alcohol is incomplete, aldehyde and irritating products being formed. Acetic acid in amounts above 10 to 12 per cent is, moreover, antiseptic to the bacteria. Below 14 per cent of alcohol, the bacteria develop readily and produce in suitable solutions, besides acetic acid, agreeable ethers which are more abundant when the oxidation is slow. Below 1 or 2 per cent of alcohol, the bacteria attack these ethers, and finally the acetic acid itself, causing complete oxidation according to the equation:

$$CH_3COOH + 4O = 2CO_2 + 2H_2O.$$

The addition of a new supply of alcohol, however, immediately arrests this reaction. In practice the acetification should be stopped when the alcohol has fallen to 1 or 2 per cent, otherwise there is a loss of flavor and of acetic acid, which may continue until all the acid is destroyed.

The length of time during which the acetic bacteria retain their vitality varies with the moisture and the temperature. In nutrient solutions, they live from one to as many as ten years; in the dry state, from three months at ordinary temperatures, to twelve months at 2°.

Processes of Manufacture

Raw Materials.—Originally vinegar was made from wine, as indicated by the etymology of the word which means "acetified wine." Later, other alcoholic beverages such as cider and beer were used for the same purpose. In these liquids, the acetic bacteria find all the mineral and organic matters necessary for their development, together with alcohol in amounts favorable for acetic fermentation. At present, a large number of materials containing alcohol, or starchy and sugary matters, which, by preliminary yeast fermentation, can be changed into alcohol are used as sources of vinegar. The most important of these are honey, malt, and various fruit juices.

All these materials make wholesome vinegar of varying degrees of quality. Those of wine and cider are usually classed as the best, and those of malt and honey next. The great bulk of the vinegar of commerce, however, at present is made by the acetification of distilled grain, potato and molasses alcohol. This is not vinegar strictly speaking but an imitation, consisting of a dilute solution of acetic acid without the various flavors which are an essential part of pure vinegar. In order to give it a semblance of the latter, it is often colored with caramel and flavored with various substances.

Other imitations of vinegar sometimes appear on the market, containing wood vinegar, or even mineral acids. These, however, are more or less poisonous and their sale, as food, is usually forbidden by law.

FERMENTATION.—If the raw material to be used is starchy or sugary, it must be first changed into an alcoholic liquid containing from 6 to 12 per cent of alcohol by volume. This is accomplished by one of the methods discussed in the preceding chapter. This alcoholic fermentation must be kept rigidly distinct from the acetification and is best carried out in a separate building. The yeast must finish its work before the bacteria commence theirs. The reason for this is that yeasts are very sensitive to acetic acid and a small quantity may paralyze their activity and prevent the change of all the sugar into alcohol, with a consequent loss of strength and quality in the final product.

The quality of the vinegar will depend on the quality of the raw material from which it is made. Wine or cider spoiled by bacterial fermentation, moldy casks, etc., will make inferior vinegar. An exception to this may be made of so-called "pricked" wines, which are simply wines in which acetic fermentation has started spontaneously. The wine or other alcoholic liquid should be perfectly clear and clean tasting and, if necessary, should be fined, filtered or pasteurized immediately before use. It should contain no antiseptic which would interfere with the development of the acetic bacteria. Sulphurous acid, if present in the free state, should be removed or oxidized by thorough aeration.

Commerical alcohols made from corn, potatoes, beets, molasses and other products can be used. The special flavors of these alcohols, due to their origin, disappear almost completely in the vinegar. This, however, is not true of denatured alcohol or that containing methyl alcohol or acetone.

The alcohol must be diluted to from 10 to 12 per cent by volume, and then made suitable for the growth of acetic bacteria by the addition of nutritive substances containing nitrogen and phosphates. This is accomplished usually by adding 10 per cent of wine, beer, malt-extract, yeast decoction, or similar material to the diluted alcohol. The waste liquids from a brandy distillery may be used instead of water for dilution. After resting a few days, the mixture is filtered and is then ready for acetification.

Before starting the acetic fermentation, it is a usual and good practice to add about 10 per cent of good vinegar to the liquid, which is thus rendered acid and therefore less liable to alteration by injurious bacteria and other microörganisms.

All the processes of vinegar-making depend on the same principle, which is to expose the liquids prepared as above to the action of acetic bacteria with full access of atmospheric oxygen at a suitable temperature. The rapidity of the process depends on the number of active bacteria present, the nutritive value of the liquid, the temperature, and especially on the free access of oxygen.

Starters and Pure Cultures.—The 10 per cent of vinegar added to the liquid to be fermented usually contains sufficient bacteria to insure a prompt start. Where this is not the case, a starter may be prepared by exposing a suitable liquid in a shallow vessel to the air of a warm room for several days. Any liquid containing about 4 per cent of alcohol, 2 per cent of acetic acid and a moderate amount of nitrogenous matter is suitable. A decoction made by boiling 50 g. of fresh yeast in 1,000 c.c. of water, filtering and adding the proper amount of vinegar and wine or beer will serve. After thorough aeration, such a liquid in a few days becomes covered with a film of acetic bacteria. This film may be used as a starter by gently submerging the vessel in which it is formed in the liquid to be acetified, or by removing with a clean sliver of wood which is afterward floated in the liquid.

In practice, such a starter gives a sufficiently pure fermentation of acetic bacteria. The particular species of acetic bacteria, however, is left to chance. Pure cultures of a particular selected form would in all probability improve the certainty of the production of good vinegar, but the method has not entered into general practice.

Apparatus.—Most metals of all kinds should be avoided as much as possible. The hoops of barrels and buckets may be protected by a coating of paraffin. Pumps may be of wood or of the special alloys already mentioned, or they may be so constructed that they will not come in contact with the liquids.

Methods

Domestic Method.—A cask of convenient size (40 to 200 liters) is fitted as illustrated in Fig. 159.

The wine or cider to be acetified, after filtering, if necessary, is poured into the cask until it is about one-half to two-thirds full, the object being to have as large a surface as possible for the growth of the bacterial film. Free circulation of air is insured by a 5-cm. hole in each head of the cask, one near the surface of the liquid and one near the top of the cask. These holes should be covered with varnished metal netting to prevent the entrance of flies.

The top bung hole is then closed with a cork, through which a funnel passes, furnished at its lower end with a glass tube extending to within a few inches of the bottom of the cask. By means of this funnel new liquid can be added without disturbing the surface film. The lower bung-hole is closed with a cork, through which passes an L-shaped glass tube which serves as an indicator of level and which also can be used to draw off the vinegar.

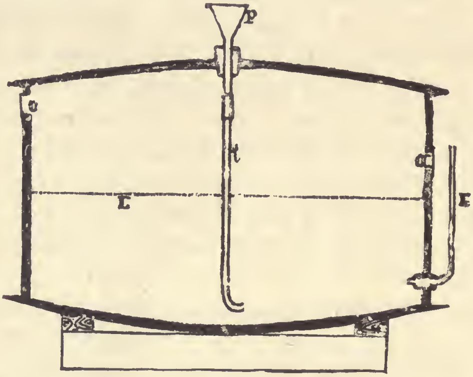

FIG. 159.—Vinegar barrel. *L*, Surface of liquid; *O*, *O*, openings for entrance of air; *t*, tube for introducing new supplies of wine without disturbing surface films; *E*, glass tube to show level of liquid and for drawing off vinegar. (*Original.*)

When this apparatus is working well, from one-fifth to one-quarter of the contents may be taken off every three or four weeks. This depends on the temperature, which should be between 10° and 18°. The vinegar drawn off is immediately replaced with wine or cider which, if added slowly, will, owing to its lower specific gravity, remain at the surface in contact with the bacterial film.

ORLEANS METHOD.—This is practically the same as the method just described with slight modifications to adapt it to large scale operations. It is the oldest commercial method and produces vinegar of the highest quality.

Barrels of about two hectoliters are usually employed, fitted essentially like that already described but with the omisson of the funnel and drawing-off tubes.

The wine is first cleared in a vinegar filter. This consists of a wooden vat filled with beech chips which have been extracted by soaking for several days in cold water. The wine remaining in contact with these chips for three or four days deposits most of its sediment.

The cask is first one-third filled with good vinegar and 10 or 15 l. of the filtered wine added. The same amount of wine is added every week for four weeks by which time the cask is half full. At the end of the fifth week an amount of vinegar equal to the wine added is drawn off and the operation repeated. The vinegar is filtered as soon as it is drawn off, placed in full tightly bunged casks and kept in a cool cellar.

Pasteur Method.—Pasteur long ago pointed out the defects of the old Orleans method and suggested improvements. The main defects of the old method are that it is cumbersome, laborious, slow and costly. There is a loss of about 10 per cent of material by evaporation and the repeated additions of liquid break the bacterial film, which then sinks to the bottom, grows anaerobically and exhausts the nutrients of the solution without producing acetic acid. These submerged bacteria finally form a large gelatinous mass which interferes with the regular progress of the operations, depreciates the quality and necessitates frequent expensive cleanings of the casks. Many attempts, more or less successful, to overcome these defects in accordance with Pasteur's ideas have been made, that of Claudon is one of the best and will serve to exemplify all.

It consists essentially of a wide, shallow, covered square vat, furnished with numerous openings near the top by which the entrance of air can be facilitated and regulated. This vat is filled to the bottom of the air vents with a mixture of four parts of good new vinegar and six parts of wine which has been pasteurized at 55° and, when necessary, filtered. On top of this liquid is floated a light wooden grating which helps to support the bacterial film and prevent its breaking and submerging during the various operations. When filled, the process is started by placing a small quantity of a good bacterial film on top of the liquid which soon becomes completely covered when the proper conditions of temperature and aeration are maintained.

Each acetifying vat is connected with a small measuring vat from which the proper amount of liquid is added every day after a corresponding amount of vinegar has been removed. These two vats constitute a unit, several of which, usually six, are united in a battery. A factory includes several of these batteries.

The batteries are fed from a large vat or reservoir, where the mixture of wine and vinegar is prepared and stored. The vinegar drawn from the batteries runs directly to filters, thence to a pasteurizer, and finally to the storage casks.

The output of these batteries is from two to five times as great per square meter of acetifying surface as that of the old method; the cost of the operation is considerably less, the loss by evaporation much reduced and the quality equal and much more under control of the manufacturer.

Rapid Methods.—In all the methods described, the surface of the liquid exposed to air, where alone acetification occurs, is small compared to the volume of the liquid. In order to hasten and therefore cheapen the process, various devices for increasing the surface in contact with air have been devised. The simplest of these is one sometimes employed in wine-making countries. The pressed pomace of red wine is broken up and placed loosely but uniformly in a tall narrow vat. In a few days, acetic fermentation commences in all parts of the mass. Wine is then sprinkled periodically on top and trickling down over the pomace, it is changed to vinegar by the bacterial film which encases every particle of the mass. The "quick" or German method of vinegar-making is based on this principle.

The apparatus used in this method consists of a tall cylindrical or slightly conical wooden vat provided with a perforated false head a few inches from the bottom and another, similar in structure, at the same distance from the top. The space between these two false heads is filled with long thin chips or shavings of beech wood which have been thoroughly extracted, first with water and then with good strong vinegar (Fig. 160). Various substitutes for beech chips have been used with more or less success. Rattan shavings and wastes are suitable; dried corn-cobs can be used but are not durable; wood charcoal in lumps is used successfully; coke is as effective as wood charcoal and more durable and is now used extensively in the manufacture of alcohol vinegar.

In operation, the liquid to be acetified is distributed over the top false head intermittently in small amounts. This intermittence of

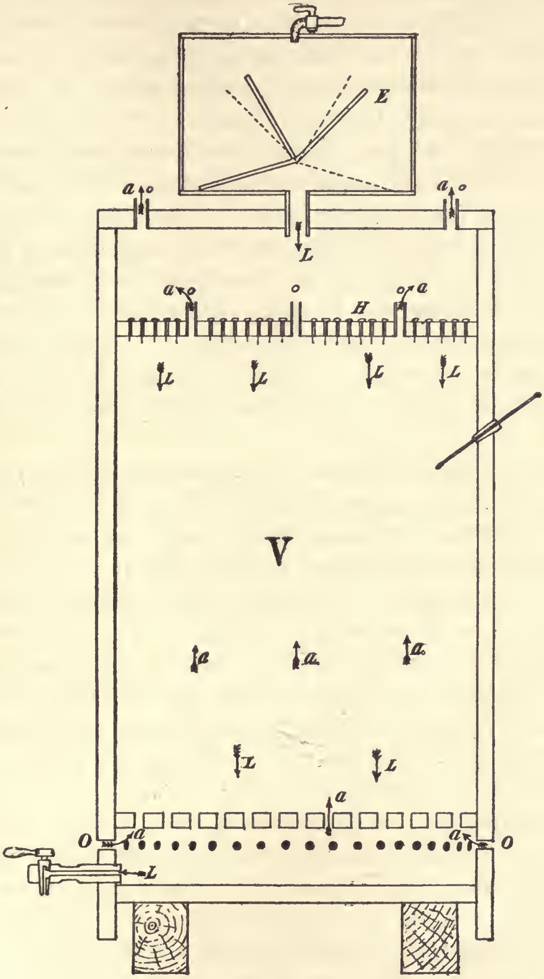

FIG. 160.—Rapid process vinegar apparatus. *V*, Mass of beech chips over which the alcoholic liquids run from *H; H*, false head with numerous small holes and threads for the slow and equal distribution of the liquid; *E*, tilting trough for the intermittent supply of liquid; o, o openings for the entrance and exit of air. ↑ Path of air; → path of liquid. (*Original.*)

supply is accomplished by various automatic devices. If the supply is continuous, the liquid tends to run in streams or currents in certain parts of the vat and much of the acetifying surface is lost; if too rapid, the bacterial film is removed from the upper part of the mass of beech chips and only the lower part is effective.

From the false head, the liquid passes through numerous small holes to the mass of beech chips, over which it trickles slowly and is acetified by means of the bacterial film which covers them. By the time it reaches the lower false head, the alcohol is in greater or less amount converted into acetic acid. Usually the liquid must pass through from two to five times or through an equal number of vats before it is completely changed into vinegar. The number of passages depends on the amount of alcohol present, the height of the acetifying column, the rapidity of the flow, the temperature, and on the perfection of the apparatus.

Oxygen is supplied by the air which, entering holes in the vat below the lower false head, passes through numerous holes in the latter, through the interstices between the chips and out through short tubes fixed in the upper false head and holes in the top. The passage of air is insured by the heating of the interior due to the fermentation. It can be regulated by the number and diameter of the air holes.

The temperature, which should be close to 30°, must be carefully regulated. If the temperature rises too high, the loss by evaporation will be much increased; if it remains too low the acetification will be retarded. Too low a temperature is less injurious than too high a temperature.

Many modifications of this method exist, having principally for their objects the more complete regulation of the temperature and air supply, the recuperation of the volatile matters, and the avoidance of the need of repassing the liquid through different acetifying columns.

ROTATING BARRELS.—Several methods are in use which attempt to combine the rapidity of the German machines with the quality of the Orleans method and which are suitable for use with wine and cider. These liquids cannot be acetified conveniently by the German method on account of the large amounts of solids and extractive matter they contain. These coat the beech chips rapidly and interferes with the perfect working of the machine.

These methods make use of a barrel filled partially or wholly with beech chips and half filled with the liquid to be acetified. By rotating the barrel at short intervals the liquid trickles repeatedly over the chips and, with proper aeration, the acetification is rapid and complete. The same principle has been applied successfully by means of "drums" 10 feet long by 4 feet in diameter partially immersed and rotating in the liquid of a closed vat to which air is admitted by adjustable holes.

FUNCTION OF THE FILM.—All these methods are based on the supposition that the formation of acetic acid depends on the work of the bacterial film at the surface of the alcoholic liquid. It seems probable that the only function of the film is to maintain the bacteria in a position where they can obtain a full supply of oxygen. If this is true, oxygen supplied by a stream of compressed air or other efficient means of aeration should be equally effective even in the absence of a film.

An observation by W. V. Cruess indicates that the film formation may actually hamper the work of the bacteria. This observation is that certain forms of vinegar bacteria which do not form films produce acetic acid very rapidly. The observation was made on wine in small flasks and at temperatures of 20°–33°. Whether the formation of acetic acid would be equally rapid in larger volumes of liquid where the penetration of oxygen would be slower has not been determined.

AFTER-TREATMENT.—Alcohol vinegars require little treatment. They should be filtered and are usually colored slightly with caramel. Being little more than dilute solutions of acetic acid without ethers or bouquet, there is no object in aging them.

Wine and cider vinegars, for the best results, require aging and careful treatment. They should be filtered and pasteurized as soon as made and stored in clean casks which are well bunged and kept constantly full in a cool place of even temperature. If too dark in color, they may be decolorized with pure animal charcoal carefully extracted with acids and water.

Before using or bottling, the vinegar should be fined with isinglass (see page 620).

DISEASES

The most troublesome pest of vinegar factories is a minute nematode, the *Anguillula aceti* or vinegar eel. It often develops around the edges of the surface of the liquid in vinegar barrels and in the acetifying columns and, if neglected, may cause putrefaction and spoiling of the vinegar. Frequent and thorough cleaning of all apparatus, pasteurization of liquids and light sulphuring of empty casks will prevent its development. The vinegar eels are easily killed by heating the vinegar to 50°. They may be removed by filtration or fining.

Microscopic mites are sometimes troublesome in neglected factories. They can be reduced by the methods recommended for vinegar eels and their entrance into the barrels or acetifying columns prevented by painting a ring of turpentine or some viscid substance around each air hole.

Vinegar flies (*Drosophylla cellaris*) are often troublesome, but can be excluded by proper screening of buildings and barrels.

Bacteria other than acetic may develop in vinegar and some of them may depreciate its quality. These have been little studied but the most harmful seem to be anaerobic forms which develop in the lower parts of the liquid protected from oxygen by the screening film of the acetic bacteria. They produce butyric acid and putrid odors and, if neglected, may completely spoil the vinegar. Sulphuring, fining, and pasteurization are the remedies.

Darkening or persistent cloudiness may be caused by oxidase as in wine and cider and is controlled in the same way. A similar defect may be caused by the tannic extractive matters of new casks or contact with iron. Aeration followed by fining will remove the cause of the trouble.

DIVISION VII

MICROBIOLOGY OF SPECIAL INDUSTRIES

CHAPTER I

SPECIAL INDUSTRIAL FERMENTED PRODUCTS

ACETONE AND ACETIC ACID*

Prior to 1914, most of the world's supply of acetone and acetic acid was furnished by the destructive distillation of wood. When wood is distilled from a retort at high temperatures it undergoes a decomposition by which acetone, acetic acid, methyl acetate, and other compounds are liberated leaving in the retort crude charcoal. The acetic acid is converted into calcium acetate by neutralization with lime. The acetic acid may be liberated by means of inorganic acids and distilled to give acetic acid or may be dried and destructively distilled to form acetone. Acetone is used extensively in explosives manufacture and as a solvent for aeroplane "dope" and many other organic compounds. It is useful also in the mixing of guncotton and nitroglycerine to form cordite, the explosive used so extensively by the British and other navies.

Two methods of producing acetone besides wood distillation came into common use during the war. One was the formation of acetone from starchy materials by direct fermentation, the Fernbach process; the other was by the formation of acetic acid by direct bacterial fermentation of sea kelp or the alcoholic and acetic fermentation of sugary material, followed by conversion of the acetic acid into acetone.

The United States Industrial Chemical Company at Curtis Bay during the war produced as much as 70,000 pounds of acetic acid per day from molasses imported by tank steamers from Cuba. Over 1000 beechwood shaving-filled generators from 10 × 18 to 18 × 25 feet in size were necessary. About 200,000 gallons of calcium acetate liquors per day were concentrated in special stills, making this plant the largest of its kind in the world.*

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* Prepared by W. V. Cruess.

In the Fernbach process corn or other grains are subjected to a carefully controlled fermentation process in which acetone is formed directly. It is not clear whether the acetone is formed from the starch or from the grain proteins—probably the latter figure to an important degree. Butyl alcohol is formed with the acetone. It is stated that 100 tons of acetone per month were produced during the war in a plant in Toronto and 250 tons per month in the United States by this process.

Bacillus macerans is used in a process, described in German patent 294683 (1914), to produce acetone from wort. Yeast is then grown in the liquid resulting in a yield of both yeast and acetone.

Mezzadroli† of the Sugar Beet Station at Rovigo, Italy, describes two organisms, *B. invertenti lattici*, and *B. invertenti acetici*, which are capable of forming lactic acid, alcohol and acetone directly from cane sugar. Their industrial use for the production of these acids from sugar waste is advocated.

The Hercules Powder Company and other companies demonstrated that acetone and acetic acid could be produced, at least under war-time conditions, from kelp by a fermentation method, first systematically investigated by L. Lieb and D. R. Hoagland of the University of California. The giant kelp of the Pacific ocean was harvested by a "mowing machine"-like device, taken by barges to the plant at San Diego, and shredded into a slimy mass of pulp and juice. This mixture was pumped into enormous tanks in which a vigorous growth of bacteria of several types developed spontaneously. The leaves of the plant and parts of the stems became liquefied. The resulting liquid containing acetic acid, ethyl acetate, ethyl propionate, ethyl butyrate, and other compounds formed during fermentation and inorganic salts was neutralized with calcium carbonate forming calcium acetate. This was treated with sodium sulphate to form sodium acetate which on concentration was crystallized and converted into acetone by a special destructive distillation process. Many organic compounds, such as ethyl butyrate, etc., were recovered from the fermented kelp liquor as valuable by-products. The Hercules plant at one time represented a $5,000,000 investment and produced about 24 tons of acetic acid or its equivalent in acetone per day from 1500 tons of kelp.

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* See H. Hibbert, Chemical and Metallurgical Engineering, 1919, pp. 397–400.

† Boll. Assoc. Ind. Zucch. e Alcool, Bologna, 1917. 142–145.

The plant has since been closed, as have others of a similar nature, which operated during the war in acetone manufacture.

LACTIC ACID*

Lactic acid is used in large quantities in the tanning industry for deliming and plumping the heavier hides. It also finds some application in other industries as well. There is, therefore, a moderate demand for the acid in commerce.

Several raw materials have been used in its preparation. The waste liquor from concrete beet silos carries about 2 per cent. of this acid and has been used direct in tanning or the liquid treated to recover the lactic acid itself. Amylaceous substances such as corn starch, barley malt, etc., are the usual raw materials, although waste milk from creameries or the whey from cheese factories may also be employed to advantage.

If starchy materials hydrolyzed with either acid or malt are used, it is possible to carry out lactic fermentation at 50° or higher and thus effectively eliminate the butyric organisms, which constitute the principal source of trouble. If milk or whey is the culture medium, *B. bulgaricus* or other vigorous lactic organism adapted to milk may be used.

The fermentation is carried out in the presence of chalk ($CaCO_3$) or zinc carbonate. Either calcium or zinc lactate is formed. The zinc lactate crystallizes satisfactorily on concentration; calcium lactate is more soluble. The salt is next decomposed with sulphuric acid and distilled, preferably in vacuo, in apparatus not corroded by lactic acid vapors.

Among the various processes described in the literature, the Friedberger† method appears to possess merit although it is difficult to see in what way his process is novel to the extent of being patentable. He uses a pure culture of *B. delbruckii* from a maltose nutrient medium which is transferred to sterile dextrose nutrient medium containing asparagin and peptone. When it has become accustomed to the dextrose, the culture is grown in a sterilized medium and the starter so produced used to ferment a sterilized liquid made by hydrolysis of any starchy material with hydrochloric acid. The fermentation takes place in the presence of sterile chalk. Near the end of fermentation the temperature may be lowered and cultures of *B. bulgaricus* may be added. It is claimed that liquids fermented in this way are of sweet odor and free from butyric acid. The liquid is treated with 0.3 to 0.5 per cent. tannin, calculated on the weight of carbohydrates originally present. The tannin unites with the albumins of the liquid resulting in clarification. The clarified liquid is filtered and decomposed with sulphuric acid in the usual way.

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* Prepared by W. V. Cruess.

† English Patent 2507, January 31, 1917.

Wehmer* reports that 15 grams of glucose will yield under average commercial conditions about 10.5 grams of lactic acid; whereas the theoretical yield is 15 grams according to the following equation:

$$C_6H_{12}O_6 = 2C_2H_4OHCO_2H.$$

The reactions involved in the purification of the acid are:

$$2C_2H_4OHCO_2H + CaCO_3 = Ca(C_2H_4OHCO_2)_2 + CO_2 + H_2O.$$

$$Ca(C_2H_4OHCO_2)_2 + H_2SO_4 = CaSO_4 + 2C_2H_4OHCO_2H.$$

The $CaSO_4$ separates as a precipitate.

Wehmer has held lactic bacteria for six years in calcium lactate formed during fermentation and suggests this method as a commercial means of storing starters of desirable strains of the organism.

Hexamethylene tetramin† has been used instead of calcium carbonate to neutralize the lactic acid slowly during fermentation and the sugar remaining is fermented with yeast for alcohol production.

Milk and whey should be sterilized before use and fermented with pure cultures of lactic organisms to minimize the constant danger from butyric acid fermentation which is favored by neutralization of the liquid with calcium carbonate.

CITRIC ACID‡

Most of the citric acid of the world's commerce is obtained from lemons, although it is possible to convert various sugars into citric acid by fermentation processes.

In California citric acid is made from cull lemons from the packing houses. Lemons are graded very closely during packing in order that the quality of the packed fruit shall be uniform and the fruit attractive in appearance. This results in a large percentage of culls which until recently were largely an economic loss. Through the investigations of Dr. E. M. Chace and C. P. Wilson of the United States Bureau of Chemistry, a commercially stable citric acid industry has been founded in California. The plant of the Exchange By-Products Company at Corona is capable of caring for over 100 tons of fruit per day. The lemons are first peeled in special rotary grating machines. The peels are distilled to recover the essential oil. The peeled fruit is crushed and pressed in powerful continuous presses. The juice is pumped into vats of about 30,000 gallons each where it is permitted to undergo spontaneous alcoholic fermentation. The purpose of the fermentation is to destroy the slimy nature of the juice, making it possible to filter the fermented juice easily. The fermented juice is filter pressed; the clear liquid is neutralized with calcium carbonate at the boiling point because calcium citrate is more insoluble in hot than in cold liquids; the calcium citrate is removed by filtration, treated with sulphuric acid in slight excess, the calcium sulphate is removed by filtration, the citric acid filtrate is partially concentrated in open vats by a stream of air, concentrated to the crystallizing point in glass-lined vacuum pans, is allowed to crystallize in shallow vats, the crude crystals are redissolved in water, and recrystallized to give citric acid of commerce. The manufacture of this product has done much to stabilize the lemon growing industry in California.

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* Jour. Soc. Chem. Ind., 1906, page 112.

† A. Pollak, U. S. Patent 1123920, January, 1915.

‡ Prepared by W. V. Cruess.

It is stated that it is possible by means of pure cultures of certain varieties of citromyces (a penicillium-like mold) if a 5 per cent. sugar solution is used, to convert 50 per cent. of maltose, 30 per cent. of sucrose, 5 per cent. of arabinose, and 24 to 29 per cent. of glycerol into citric acid. Wehmer states that citromyces cultures may be easily obtained by exposing a 2 to 5 per cent. solution of citric acid and cane sugar to the air for several weeks. These cultures may be used to convert dilute cane sugar solutions containing a small amount of ammonium nitrate, dipotassium hydrogen phosphate and magnesium sulphate into dilute solutions of citric acid which may be safely used as lemonade or a source of citric acid. As high as a 4 per cent. citric acid solution may be readily attained at 18° to 25° in 8 to 14 days. If left too long the acid is oxidized and the liquid in time becomes neutral or alkaline. If calcium carbonate is added before inoculation, higher yields of acid are obtained. Above 25° growth is inhibited with many strains of citromyces.

The reaction is of an oxidizing type but the exact set of chemical reactions that take place is not well understood. It is not a simple oxidation process because citric acid possesses a branched side chain not present in the sugar molecule. For each 50 grams of dextrose converted into 13.3 grams of citric acid, 10 liters of oxygen is required according to Wehmer.

C. citricus has given very high yields of acid, although *C. Pfefferianus* and *C. Glaber* have also given good results. *Sterigmatocystis nigra* also forms citric acid in dilute sugar solutions. Many forms have been studied and described but in practically all cases other acids and compounds than citric acid are formed, making the recovery and purification of the citric acid very difficult or impossible.

Most of the citromyces that have come to the writer's attention form during the first stages of growth a cottony white mycelium. This usually later turns to pale green and in time olive brown; some cultures remain permanently white. Citromyces may readily be mistaken for *Penicillium expansum*, an organism which tends to contaminate cultures of citromyces if precautions are not taken.

It is possible that the citromyces fermentation may in time be used for commercial purposes, but it is at the present time in the experimental stage.

White Lead*

Basic acetate of lead used so extensively in paint is of finer grain and better covering quality if made by the Dutch or fermentation process than if made by purely chemical processes. Grids of pure lead are stacked between tiers of spent tan bark; several layers of lead and tan bark being built up in well-insulated rooms. Acetic acid and CO_2 are formed. The acid fumes rise from the heat of fermentation and combine with the lead plates, forming a crust of white lead. Most of the lead is converted to the basic acetate.

The exact nature of the fermentation is not well understood. It is probably not of the usual "alcohol-acetic" type carried out by *S. ellipsoideus* or *S. cerevisiæ* and *Bact. aceti*, but is possibly a mixed fermentation of several types of bacteria. Various substitutes for tan bark have been tried but Californian manufacturers at least have found the spent bark by far the most suitable material.

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* Prepared by W. V. Cruess.

LEATHER*

In the manufacture of leather, bacteria play a very important and extremely interesting rôle. Success depends to a very great degree upon the proper control of microörganisms during the various steps of the manufacturing process.

Most hides and skins are received at the tannery fresh with more or less adhering blood and flesh. Hides from outlying districts or foreign countries are received in the salted or dried states.

The hides are first placed in water, in the so-called "soaking pits," to remove the blood from fresh hides, the salt from salted hides and to plump and soften the dried hides. Formerly "putrid soaks" were used. The liquid in these soaks had stood for a long enough period to become swarming with putrefactive organisms. Much of the gelatin of the hide was dissolved and often the grain of the hide was injured by bacterial action. *B. fluorescens liquefaciens*, *B. megatherium*, *B. subtilis*, *B. proteus vulgaris*, *B. proteus mirabilis*, were commonly found in this liquid according to J. T. Wood.† None are beneficial to the hide and most of them are harmful. If the water is changed frequently there is less danger of putrefaction; Proctor recommends the use of an antiseptic solution of 1:1000 sodium hydroxide or from 1 to 3 per 1000 of sodium sulphide to prevent bacterial growth during soaking.

The wool is often removed from sheep skins by bacterial action. The hides are hung on racks in an air-tight room at 15° to 20°. Wood reports the following organisms to be active during this "sweating" process: *B. fluorescens liquefaciens*, *Bact. pilline*, and a streptococcus. These organisms secrete enzymes which dissolve the cementing substance at the roots of the wool permitting the wool to be easily removed. It is difficult to check or control this process; usually some hides from the "sweating stove" will be spoiled by too prolonged putrefaction. Caustic sodium sulphide is used to a considerable extent to replace the sweating process.

The hair is removed from hides and calf skins by liming. The hides are first placed in a lime pit consisting of water and slaked lime that has been in use for some time. This old lime pit contains proteolytic bacteria which develop upon the gelatin and other nutrient material dissolved from the hides previously treated. These organisms exert an appreciable depilatory action and considerably increase the activity of the lime. Some of this effect is due to ammonium salts and amines, formed by the bacteria. Much of the bacterial effect can be duplicated by adding ammonium sulphate. From this lime pit hides in time go to pits containing new lime and thence to the beam where the hair is removed by scraping.

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* Prepared by W. V. Cruess.

† Wood, J. T: Bacteriology of the Tanning Industry, Journal of Soc. Chem. Ind., 1910, 666.

If the hide is for sole leather or other heavy leather it goes direct to a dilute lactic acid or dilute mineral acid bath where the excess lime is dissolved without removal of any appreciable amount of the hide substance needed to give rigidity to the leather.

Soft leathers require a different treatment. Fine leathers such as glove leather are usually given a puering treatment in which the skins are placed in a dilute infusion of dog dung which has previously been permitted to stand several days to develop the proper types of bacteria. Other skins are given a bating process in pigeon or hen dung infusion or in a proprietary bating solution. The hen or pigeon dung is prepared for use by soaking in warm water several days. Fermentation and vigorous development of bacteria ensue. The fermenting infusion is diluted with water and mixed with the skins in the "bating wheel" which consists of a large wooden paddle wheel revolving in a tank of the bating liquor. In the puer and bate liquors the lime is dissolved from the hides to some extent by lactic acid and also through the action of amines and ammonium salts formed by the bacteria from proteins of the skins. Some of the intra-cellular substance of the skin is dissolved. The skins become soft and pliable, *i.e.*, "fall." The surface becomes slippery and the skin retains the imprint of the finger if pressed between the thumb and finger. Too prolonged bating or puering results in pitting of the hide; in fact, it is possible to cause the hide to go completely into solution by several days' bating at 37°, the temperature ordinarily used in practice.

Many attempts have been made to replace the dung infusions with pure cultures. In coöperation with F. H. Wilson the writer isolated a number of organisms from bate liquors. Most of these were of the colon group; some of the proteus group. The colon group of organisms gave good results in pure culture when grown in dilute milk (diluted 1:10) with water or in dilute sugar solutions. The milk or other sugars used protected the skins against overbating and injury but at the same time permitted effective removal of the lime and very satisfactory bating or softening of the texture. Similarly, good results were obtained by Noble at the University of California by using pure cultures of *B. subtilis* in hay infusions or other cheap and suitable liquids. The practice which he preferred was to heat an infusion of hay to boiling and to allow this to stand until a good growth of *B. subtilis* had developed.

Patented mixtures of a pure culture of *Bacillus erodiens* or other suitable organisms and a suitable nutrient medium in dry and soluble form have been successfully employed to replace dung bates. A mixture of pancreas extract and ammonium sulphate has been sold under a trade name and used with fair success. It therefore appears that the use of the dung infusions is not essential.

At one time the skins from the bating liquor were transferred to a fermenting infusion of bran known as the "drench" for 18 to 24 hours. Acid formed by *B. furfuris* and similar organisms removed the last traces of lime. At the present time a "pickle" consisting of dilute sulphuric acid and sodium chloride is commonly used to remove the lime left in the skins from the bate liquor. The calcium sulphate so formed crystallizes in the pickle vats as a hard incrustation.

The skins are now ready for the tan pits. They enter the old and more dilute tan liquors first. In these the bacteria carried over on the hides from the bate, etc., adapt themselves. Some lactic acid is formed and tends to plump the skins and facilitate the penetration of the tannin. Dilute tannic acid solutions must be used at first to permit deep penetration of the tannin. The concentration of tannin is increased in the succeeding pits until a saturated solution is reached. The extractive matter from the tan bark in the dilute liquors supports a varied growth of lactic bacteria, yeasts and molds, while sufficient tannin is present to check effectively the growth of putrefactive organisms. Tan bark is used for ordinary leather while sumach and other light colored tannin extracts are used for lighter colored leather.

Tannin may be replaced by chrome alum or potash alum, or other mineral tanning materials. Some of the toughest and most resistant leathers are of this type.

INDIGO*

Indigo is now for the most part made synthetically. This dye was formerly made from certain species of *Indigofera*, principally *I. tinctoria*. This plant contains a glucoside, *indican*, which by fermentation and oxidation yields *indigo*.

The plants are placed in water at a temperature of 25° to 35° and undergo a spontaneous alkaline fermentation which splits up the indican into a sugar (*indiglucin*) and *indigo white* which remain in the solution. This solution is then thoroughly aerated and the indigo white oxidized into *indigo blue* which is insoluble and forms a sediment. This sediment is dried and constitutes the old indigo of commerce.

Many bacteria are found in the fermenting liquid, but the cause of transformation has been shown to be a specific form, *Bacillus indigogenus*, closely related to Friedländer's pneumonia bacillus. It is strongly aerobic and surrounded by a gelatinous envelope.

RETTING*

The separation of the fibers of flax, hemp, ramie and similar plants is brought about by a complex spontaneous fermentation. The plants are either left on the surface of grassy meadows exposed to alternate wetting and drying or immersed in water. In either case, the tissues are gradually disintegrated by microbial action, more rapidly in the wet process.

The fermentation, principally bacterial, is due to many species. Several have been described as being the principal agent in the process but it is probable that the effects are due to the united action of several, both aerobic and anaerobic.

Among the forms to which the retting has been attributed are *B. amylobacter* of van Tieghem, an anaerobic form which attacks the pectic matters and to some extent the cellulose. *Granulobacter pectinovorum* of Beyerinck and van Delden, also anaerobic, transforms the pectic matters into sugars which it decomposes, producing butyric acid. Many other forms have been described and part of the work has been ascribed to *Mucor*, *Penicillium*, and various molds.

Cultures of *Granulobacter pectinovorum* and other forms have been successfully used to hasten the process.

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* Prepared by F. T. Bioletti.

DIVISION VIII

MICROBIOLOGY OF DISEASES OF MAN AND DOMESTIC ANIMALS

CHAPTER I*

METHODS AND CHANNELS OF INFECTION

INFECTION DEFINED

The term infection implies the entrance of animal or vegetable organisms into the body of another animal or plant, their multiplication and their injury to that body. In most instances the organisms enter the tissues of the animal or plant body, although this is not true in every case of infection. It is possible in certain instances to produce the symptoms of an infection by introducing into the body the chemical products elaborated by some pathogenic organisms. For example, the injection of tetanus toxin into the body causes the typical symptoms of tetanus to result. Tetanus toxin is made by growing *B. tetani* in beef broth under anaerobic conditions and filtering out the bacteria by passing through porcelain filters. These chemical products do not occur naturally unassociated with the pathogenic organisms and therefore they do not produce infections when artificially injected in the usual sense.

The disease-producing organisms with which we will especially concern ourselves in the subsequent discussion are those which are very minute in size and are of three kinds: first, bacteria; second, protozoa; and third, ultramicroscopic microörganisms or viruses.

It is essential to have clearly in mind what is meant by an infectious disease and a contagious disease before entering into any detailed discussion, although some authorities attempt to make no distinction. An *infectious disease* is any disease produced in the plant or animal body which is due to a foreign animal or plant organism. The name is applied to the nature of the cause of the disease. A *contagious*

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* Prepared by E. F. McCampbell.

disease is an infectious disease which is transmitted from one individual to another by contact. The term refers to the method of transmission rather than to the cause of the disease. It is possible that certain contagious diseases may be transmitted by indirect contact or by the agency of fomites but many authorities now hold the view that these factors are non-essential and that most contagious diseases are transmitted by direct contact.

Microörganisms of Disease Considered and Classified

Pathogenic Bacteria.—Bacteria which produce disease are known as *pathogenic bacteria*. Of the many thousand species of bacteria only a comparatively few species have anything to do with the diseased processes in the plant or animal body. Those bacteria which are capable of growing in the body of animal or plant may be designated as parasitic bacteria. Some bacteria can grow only in the animal or plant body and do not exist for any period of time outside of it. They are known as *obligate parasites*. There are others which may produce disease in the animal or plant body which can grow and reproduce outside the body. They are known as *facultative saprophytes*. There are still other bacteria which ordinarily live outside the animal and plant body and which exist largely upon dead organic material, which when taken into the body occasionally produces disease processes. They are called *facultative parasites*. As an example of an obligate parasite the *Bact. lepræ* of leprosy may be cited, although in this instance certain observers have claimed to have cultivated the bacillus in pure culture. However, the results are not in any sense uniform. Improved bacteriological technic has made possible the cultivation of a large number of bacteria which heretofore were regarded as obligate parasites. As examples of facultative saprophytes the *B. typhosus* of typhoid fever and the *Msp. comma* of cholera may be mentioned. As examples of facultative parasites *B. tetani* of tetanus and *Bact. welchii* of gaseous gangrene may be mentioned.

Pathogenic Protozoa.—There are several infectious diseases in man and animals which are caused by pathogenic protozoa. Among the common diseases due to protozoa there may be mentioned malaria, syphilis, rabies (the nature of the organisms involved in syphilis and rabies is not well understood however), amœbic dysentery, Texas fever, infectious jaundice of dogs, and the various trypanosome infections, such as sleeping sickness, nagana, dourine, and mal de caderas. It is difficult to cultivate artificially the pathogenic protozoa outside the animal body in pure culture. The *Trypanosoma brucei* of nagana and the *Trypanosoma lewisi* of the rat have been cultivated. The *Entamœba coli* and the *Entamœba tetragena* of dysentery, the various types of the *Plasmodium malariæ*, and the *Treponema pallidum* of syphilis have also been cultivated, and it is stated that under certain conditions the *Piroplasma bigeminum* of Texas fever may be artificially grown.

Ultramicroscopic Microörganisms or Viruses.—There are some infectious diseases the causes of which have never been discovered. The infectious agents in most instances cannot be cultivated and cannot be stained by the ordinary bacteriological methods. The presence of ultramicroscopic organisms has been demonstrated in several ways. For example, when the ordinary bacterial culture is run through a fine porcelain filter, the filtrate contains no microörganisms and consequently when inoculated into animals is non-infectious, although if soluble toxins be present there may be evidences of an intoxication. When the viruses or the infected body fluids of men or animals suffering from the diseases mentioned below are passed through a fine porcelain filter the filtrate remains infectious, therefore demonstrating that the viruses or microörganisms are filtrable and are probably so small that they cannot be seen. Examples of diseases due to agents belonging to this class are as follows: hog cholera, yellow fever, foot-and-mouth disease, rinderpest, epithelioma contagiosum of fowls, chicken typhus, horse sickness, acute poliomyelitis, etc. There are several infectious diseases of unknown cause, the viruses of which are not filtrable; for example, smallpox, cowpox and vaccinia, typhus fever and Rocky Mountain spotted fever. There are still other diseases of unknown cause about which nothing is known regarding the filterability of the etiological agents of the disease. Scarlet fever, chickenpox and measles belong to this class. These diseases can be inoculated into animals only with great difficulty and the virus cannot be cultivated or secured in sufficient quantities from the experimental animals for study. A possible explanation of some of these diseases of unknown cause may be found in the proposition that two microörganisms may each produce non-toxic substances, and that when these non-toxic substances come together, a toxic substance may be produced. This condition of affairs might explain certain infectious diseases in which microörganisms are known to occur, and in which they cannot be directly connected with the disease as causative factors. For example, the *Strept. pyogenes* very frequently occurs in both scarlet fever and smallpox. It has been shown absolutely that this organism is not the cause of these diseases, but there is a remote possibility that it may act in the so-called associative relation with some other microörganism or virus, as mentioned above, and produce the typical symptoms of these diseases. It has been recently stated that scarlet fever is due to a filtrable virus but there is every reason to believe that the occurrence of the *Strept. pyogenes* materially changes the character of the infection and makes it more severe. The associative relationship of infectious organisms is probably not the logical explanation for all infections of this character. It might be mentioned in this connection that the view is held by some investigators that some of the infectious diseases of unknown etiology are due to enzymes and that a so-called autocatalysis explains the seeming reproduction in the body of the viruses. This theory is, however, without substantial proof.

The Distribution of Pathogenic Microbic Agents in Nature

The causal microörganisms of most of our infectious diseases are found principally in the bodies of diseased man and animals. There are some exceptions to their being found only in the bodies of the diseased. Notable examples are found among certain of the wild animals such as the brush-buck, wildebeast and others which serve as reservoirs for the microörganisms of some of the most fatal of protozoal diseases. These animals seem to be naturally immune. Various insects which are factors in the transmission of certain infectious diseases do not suffer from these diseases in any form and are naturally immune. The most common source, however, is the diseased animal or human body. There is no doubt, for example, that the natural habitat of the *Bact. diphtheriæ* is in the throat and nasal passages of persons suffering from or convalescing from diphtheria. Occasionally these bacteria are also found in the nasal passages and throats of persons who have never had diphtheria. The same is true of the *M. intracellularis var. meningitidis*, of cerebro-spinal fever. The *B. typhosus* of typhoid fever also has its natural abode in the intestinal tract of persons suffering from or convalescing from the fever. The same is true with the majority of the causal microörganisms. There are some microbic agents, however, which exist in the soil but probably do not undergo multiplication such as the *B. tetani* of tetanus or lockjaw, *Bact. Welchii* of emphysematous or gaseous gangrene, and the *B. botulinus* of meat poisoning. These bacteria sometimes exist in the intestinal tracts of animals such as the horse and in all probability their occurrence in the soil is due to their deposition in manure.

THE OCCURRENCE OF PATHOGENIC MICROBIC AGENTS UPON AND IN THE BODIES OF HEALTHY ANIMALS AND MAN

The exposure to the air of the external surfaces of the body, of course, makes it especially easy for microörganisms to collect upon them. The large percentage of the microörganisms which collect on the external surfaces are non-pathogenic but there are frequently disease-producing ones among them. The various varieties of the *M. pyogenes* are almost universally present on the skin and also on the exposed mucous membranes. *Strept. pyogenes*, *Bact. influenzae*, *Bact. tuberculosis*, *M. intracellularis var. meningitidis*, *Strept. pneumoniæ*, *Bact. diphtheriæ* and many other species may be present. The mouth and nose are excellent places for microörganisms to collect and excellent for their growth as the requisite conditions such as food, heat and moisture are present. It has been stated on competent authority that all the species of bacteria which have been described as occurring in various parts of the body have also been found in the mouth. These bacteria do not necessarily produce disease or injure the body unless the vitality is lowered and they enter into the tissues. They feed upon the desquamating cells and the excretions. It is exceedingly interesting to note that *Bact. tuberculosis* and *Bact. diphtheriæ*, as before stated, have been found in the nose of persons who have never had these diseases. These bacteria have also been shown to be virulent and undoubtedly such persons are extremely dangerous to other more susceptible persons. It is also frequently noted that pathogens are found in the bodies of persons after they have recovered from the disease and that these individuals disseminate the microörganisms and infect non-immune individuals. This may be the case in diphtheria, epidemic meningitis, typhoid, Asiatic cholera and dysentery "bacillus carriers."

In regard to the occurrence of microbic agents in the internal organs of the body the following may be said. For a long time it was claimed that the internal organs of man and animals were sterile. Neisser is one authority for the statement that the internal organs of healthy animals are sterile. This has been shown not to be the case universally. Experiments have shown that fifty per cent of the internal organs of rabbits, guinea-pigs, cats, dogs, mice, horses and cattle are not sterile. *Bact. tuberculosis* has been found in absolutely normal human and bovine lymph glands. The various pus-producing micrococci have been frequently found in the spleen, kidney, liver, etc. Perhaps the commonest group of bacteria to be isolated from the internal organs are the intestinal forms. It has been demonstrated that intestinal microorganisms invade the tissues with surprising rapidity when for any reason the resistance of the body is lowered. It has been noted also that there are more bacteria in the internal organs of animals which have been fasted than in those which have been fed. Peristaltic action and the diffusion of food through the intestinal wall may be influencing factors. The fact that the internal organs are not sterile in every case is important as it may account for the so-called autogenic infections.

THE MANNER IN WHICH INFECTIOUS AGENTS ENTER THE BODY AND THEIR SOURCES

Air-borne Infections.—The causal microörganisms of infectious diseases are frequently excreted from the body of the diseased individual and are deposited on the clothing, furnishings, on the floors and walls, or on the ground. These microörganisms probably do not proliferate except in rare instances, but frequently remain virulent for a short period of time and are capable of being carried through the air for short distances, producing in certain instances disease in other individuals. There is no doubt that in diseases such as smallpox, measles, scarlet fever and other acute exanthematous diseases together with such diseases as plague and diphtheria, that the infectious agents may be carried through the air after having been deposited on clothing and furnishings. However, recent investigations have shown that this method of transferring infection is comparatively rare and that most infections are transmitted by direct contact.

In the beginning it was supposed that the only way that bacteria could be carried in the air was after having been dried on particles of dust and carried by currents of air. This, however, has been shown not always to be the case and we now know that infectious microorganisms may be carried on small particles or droplets of sputum or moisture. These two types of aerial infection are known, respectively, as dust and droplet infection.

Dust Infection.—Infectious microörganisms to remain virulent and be able to produce infection must be able to successfully resist drying after being affixed to particles of dust. After being dried the particles are frequently moved and whirled about by air currents. The larger particles of material quickly settle down but the small, almost invisible pieces of dried material may remain suspended for three or four hours. It is these small particles which are usually inhaled or deposited on the skin and mucous membranes of normal individuals that produce infections providing the microörganisms have not been killed by drying or exposure to sunlight. *Bact. tuberculosis* is sometimes carried in this way as well as certain other pathogens. The fact that small-pox virus remains active after drying indicates, at least, that dust containing it may be infectious. The extent of such dissemination is quite limited.

Droplet Infections.—It has been demonstrated that during the processes of talking, coughing, and sneezing, small bubbles or droplets of sputum are thrown out into the air. These particles remain suspended for some time and may be inhaled or deposited elsewhere. It is surprising the distance that these small particles may be carried. It is stated that they are frequently thrown out thirty feet or more. It has been shown that *Bact. tuberculosis* is rarely thrown out over four or five feet by the cough of the tuberculous individual. It should be remembered that these bacteria will remain alive two to three weeks when in the dark but that they live only a few hours when exposed to the sunlight. The pathogenic microörganisms or viruses which are commonly disseminated by droplets of moisture are those of whooping cough, mumps, measles, influenza, epidemic meningitis, pneumonia, and pneumonic plague.

Air-borne infections rarely occur, as previously stated, and are not of great importance in the open air where sunlight has free access to the disease germs but this type of infection sometimes occurs in crowded quarters such as dark shops, schools, tenements and railway trains. However, the factor of direct contact must be given its due weight in such instances.

Water-borne Infections.—Pure infections of this type occur in practically only five diseases, namely, Asiatic cholera, typhoid fever, paratyphoid fever, and in dysentery of the amœbic and bacillary forms. The length of time that these microörganisms will remain alive in the water depends on the quantity and quality of organic matter present. Only under rare circumstances do bacteria proliferate in water of very high organic matter content. Ordinarily microörganisms will live only a few days if the water is absolutely pure. They have, however, been known to live for several weeks in ordinary river water. The drinking of water or of fluids or material contaminated by water is the common but not the only way these diseases are acquired.

Infections from Soil.—The soil as a source of infectious microorganisms is of importance in only a few diseases, namely, anthrax, tetanus, symptomatic anthrax, malignant edema, emphysematous gangrene, Asiatic cholera, and typhoid fever. In the first five mentioned infection always takes place through some wound usually in the skin and in the last two diseases mentioned infection is usually through the intestinal tract but may also occur by means of wounds. The microörganisms of anthrax, tetanus and emphysematous gangrene, or more specifically the spores, will remain in soil for long periods of time. They are sometimes found in the active vegetative stage but it is probable that they do not proliferate to any extent in the soil. They exist as ordinary saprophytes. The microörganisms of typhoid and cholera have been known to remain alive for a year or more in soils containing large quantities of organic matter. The various pyogenic micrococci are also occasionally found in the soil and may enter the body of man and animals through wounds. These last-mentioned organisms may live for indefinite periods of time on the skin and enter the body only when the resistance of some tissue is lowered.

Infection from Food.—Quite a large variety of pathogenic microorganisms have been found in the various food products. Milk is perhaps the most common food product to be infected. The causal agents of diphtheria, scarlet fever and some other diseases have been disseminated by means of milk. Milk contaminated by water containing *B. typhosus* may be the means of conveying typhoid fever, and the dissemination of Malta fever is accomplished by the drinking of the milk of infected goats. Typhoid fever has also been known to have been acquired from the eating of vegetables which have been washed in water containing the pathogens. Oysters and various shell fish have been known to carry the microbic agents of typhoid fever and Asiatic cholera. Three infections coming from meat and certain other foods sometimes occur, namely, botulism, enteritis and occasionally paratyphoid fever. In these instances the causal microörganisms are in the meat. Another type of infection known as ptomain poisoning also occurs from the eating of meat or fish which has been acted upon by saprophytic bacteria and the proteins split up into toxic substances.

Animal Carriers of Infection.—Animals may communicate disease microörganisms to one another and to man in three ways, namely, first, by direct or indirect contact; second, by serving as mechanical carriers from one individual to another; and third, by serving as intermediate hosts for the microbic agent and then subsequently communicating it to another. As examples of the first proposition, the fact that tuberculosis has been communicated from cattle to man, that glanders has been communicated from horses to man, and that anthrax has been communicated from sheep to man by contact may be mentioned. It has also been stated that the cat, while not suffering from true diphtheria, seems to be able to transmit this infection and the dog may also transmit rabies to the man. In the second method of transfer, the mechanical carrying of an infection, the insects are principally concerned. It is well demonstrated that common flies frequently carry *B. typhosus* on their feet from the infected patient or the excreta and deposit them on the food materials thus causing infection when the food is eaten. The various suctorial insects also may suck up the blood of one individual and carry the infectious agent to the normal individual. Notable examples of this are found in the transmission of the various trypanosomiases by the tsetse and other tropical flies, and of Rocky Mountain spotted fever by the wood tick. The same is true of *Bact. pestis* of plague which is carried by the flea, of Texas fever by the cattle tick, and it has been shown recently that the louse may be one of the agents in the transmission of typhus fever. As an example of the third method, the serving as an intermediate host and the carrying of the causal agent, the mosquitoes which serve as the only means of transmission of the causal microorganisms of malaria and yellow fever and in which these parasites pass a certain cycle of their existence, may be mentioned.

Human Carriers of Infection.—It has been mentioned previously that man is capable of carrying infectious agents when he himself is not infected. For example, in the case of diphtheria it has been repeatedly shown that convalescents from diphtheria, persons who have had the disease, and persons who have never had the disease, frequently carry the etiological microörganisms of this disease in a virulent form and are accordingly exceedingly dangerous as disseminators. Not uncommonly persons who have had typhoid fever carry large numbers of virulent *B. typhosus* in their bodies, particularly in the gall-bladder, and disseminate them through the intestinal excreta thus causing many infections when this excreta comes into contact with water used for drinking purposes or food supplies. *B. typhosus* may be carried for many years. Asiatic cholera is occasionally carried in the same way. It has also been shown that well individuals may carry the etiological agents of epidemic cerebrospinal meningitis and acute poliomyelitis or infantile paralysis. Individuals who carry infectious organisms are popularly known as "bacilli carriers" and should always be kept under rigid quarantine or observation.

Contact Infection.—It is only necessary to emphasize certain points in addition to what has been said in the foregoing. It has been stated that animals may communicate an infectious agent to other animals of the same or different, but susceptible, species by direct contact. Probably the commonest diseases to be communicated by animals to each other are tuberculosis and glanders. This is commonly accomplished by the rubbing of the mouths and noses together although the disease may be acquired in other ways. Among the human race the diseases which are usually communicated by the contact of one individual with another are diphtheria, scarlet fever, smallpox, chickenpox, mumps, measles, gonorrhea, chancroids and syphilis. In the six first mentioned diseases it seems that the expirations and possibly in rare instances the desquamations of the skin in those which have an eruption carry the causal microörganism. The infectious agent is inhaled into the nose or throat. Some of these diseases may be in rare instances transmitted by intermediate agents, clothing, etc. (fomites). In the last three diseases mentioned, which are known as the venereal diseases, an abrasion of the integument is a prerequisite and the infectious agent must enter by this route. Infection is usually brought about by absolute contact of one individual with another.

In leprosy also almost direct contact is necessary for a transfer of the infectious agent.

THE ROUTES BY WHICH INFECTIOUS MICROÖRGANISMS ENTER THE BODY

Microörganisms enter the body through either the external or internal surfaces. It has been shown that the absolutely healthy and intact skin furnishes an efficient barrier to the entrance of infectious agents. Pathogenic bacteria, for example, the streptococcus and the various varieties of the staphylococci, are present on the skin almost continuously yet do not often produce infections. When there is an abrasion of the skin or a diseased duct or hair follicle the bacteria frequently pass down into the skin and an infection results. When the pyogenic bacteria, such as mentioned above, are vigorously rubbed into the skin infection sometimes takes place but in this instance also there has been some mechanical injury to the skin. Minute unobserved abrasions of the skin also serve commonly as points of entrance for the *Bact. pestis* of the plague. The microörganisms of tetanus, anthrax, symptomatic anthrax and malignant edema always enter the skin through wounds. Sometimes the infectious agents remain local and at other times are carried from the point of the original entrance. This may take place in different lengths of time. For example, in tetanus the bacteria remain localized in most instances at the point of the original wound and their toxin diffuses from this point. In the various pyogenic infections the bacteria usually remain localized. However, in anthrax the bacteria are carried into the circulation in a very few minutes after they enter the wound. In the newborn infection very frequently enters the body through the umbilicus or navel. Tetanus is one of the common diseases acquired in this way in certain localities. Microörganisms may also enter the skin through the wounds made by insects such as mosquitoes, flies, ticks, and fleas. The larger and the clearer cut the wound the less the danger of infection because of the mechanical and bactericidal barrier of the fibrin and the bactericidal action of the blood serum. A free flow of blood also washes the microörganisms out of the wound. Crushing wounds are especially dangerous inasmuch as there is not a free flow of blood and also there is a good chance for the growth of anaerobic bacteria such as those of tetanus, malignant edema, symptomatic anthrax and emphysematous gangrene.

The mucous membranes of the nose, throat and mouth are quite resistant to infection. The epithelial coat, the mechanical action of the mucus and saliva and possibly the slight bactericidal action of the saliva are the barriers. Infections of the thin non-resistant mucous membranes of the new-born do occur and necrosis sometimes results (noma). The mucous membrane of the mouth and throat is frequently the seat of primary infection when it is injured. The actinomycotic fungus usually enters a lesion in the mucous membrane made by straws and other substances. The ducts of the salivary glands also serve as points of entrance for certain infectious agents. The tonsils are very commonly the seat of infections especially with the *Strept. pyogenes* and *Strept. pneumoniæ*. Septicemias, as for example those occurring in diphtheria, and especially in scarlet fever, frequently arise from infection of the tonsils with *Strept. pyogenes*. These structures are also the primary point of invasion in cases of acute rheumatism and possibly in certain cases of pulmonary tuberculosis. The nasal mucous membrane is undoubtedly more permeable to infectious agents than that of the oral cavity. The microörganisms of acute epidemic meningitis, acute poliomyelitis, measles, leprosy and glanders undoubtedly most frequently enter the body through lesions in the membranes of the nose. Infection may be carried into the nose directly or pass from the conjunctiva through the naso-lachrymal duct.

The flora found in the eye is quite extensive. The conjunctiva is frequently the seat of primary infections. The pyogenic cocci and the *M. gonorrhoeæ* are among the common infecting agents. It is possible that certain points of infection are provided by the conjunctiva being injured by dust particles. The tears are not bactericidal and only serve to mechanically wash the eye. Infections of the conjunctiva are frequently very severe. There is no doubt also that other pathogens are caught in the eye and washed into the nose where they set up infections or are carried through the membranes to set up infection elsewhere. *M. intracellularis var. meningitidis* of epidemic meningitis is known to pass in this way and possibly *Bact. pestis* of the plague in certain instances.

Infectious microörganisms after being taken into the body through the nose or the mouth may either pass to the lungs through the trachea or down the oesophagus to the stomach and intestines. During the ordinary inspiratory part of a respiration it is probable that microorganisms cannot pass directly into the alveoli of the lung as the tortuous passage, the mucus and the cilia are fairly efficient barriers. Bacteria may be inhaled directly into the finer bronchi and the alveoli during forced inspiration such as that attendant upon hiccoughing and sneezing. Infections of this kind occur in pneumonia, tuberculosis, and influenza. Microörganisms frequently lodge on the membrane of the trachea and are here taken up by the leucocytes and carried to the lungs, bronchial lymph glands, and occasionally to other parts of the body by the blood and lymph. It is probable that such a form of infection occurs sometimes in pneumonia, tuberculosis and plague.

Infectious microörganisms very frequently pass down to the stomach and intestines. The mucous membrane of the stomach is normally very resistant to infection due to the hydrochloric acid which is normally present in the gastric juice and which in normal amount is distinctly antiseptic. It should be remembered that in instances where the acidity of the stomach is lowered that microorganisms will develop. All toxins with the exception of that of *B. botulinus* of meat poisoning are destroyed by the gastric juice. The intestines are less resistant to infection. It is here that the causal microörganisms of typhoid fever, Asiatic cholera, chicken cholera and dysentery and the various hemorrhagic septicemias find their particular affinities. These bacteria enter or attach themselves to the intestinal wall and in the case of cholera and dysentery this is the only point of infection. The *B. typhosus* has occasionally been known to enter at other places. This bacterium, however, commonly localizes in the lymphatic patches (Peyers) of the intestine, and may enter the blood from this point. It should be noted that some bacteria can pass through the wall of the intestine when it is seemingly intact. This point has been repeatedly demonstrated in the case of *Bact. tuberculosis.*

The genital organs of the male and female are susceptible to infection with microörganisms in certain instances. The *M. gonorrhœæ* of gonorrhœa and the *Treponema pallidum* of syphilis find their usual portals of entry in the genital tract. They have, however, been known to infect other parts of the body as the mouth, rectum, and the conjunctiva. The etiological bacteria of gonorrhœa can penetrate the seemingly intact male urethra but not the vagina of the female on account of the bactericidal secretion and the character of its squamous epithelium. Other bacteria, as for example, the *Strept. pyogenes*, *M. pyogenes var. aureus* and *B. coli* are sometimes found infecting the genital tract in cases of chronic urethritis.

The kidneys, ureters and bladder are sometimes infected but usually the infecting agent is carried in the circulation although it occasionally ascends through the urethra from without.

In conclusion, the proposition of germinal and antenatal infection must be mentioned. By true germinal infection is meant the carrying of the infectious organisms of a disease by the ovum or the spermatozoa and its incorporation in the development of the embryo and fetus. It is doubtful if this ever occurs. Some authorities claim that it is possible and that it has been demonstrated that the spermatozoa may carry the *Treponema pallidum* of syphilis, but this is not generally accepted. Antenatal infection or infection of the fetus before birth does occur. Infectious organisms enter the fetus only as a result of intimate contact with the mother and it has been repeatedly shown that tuberculosis and syphilis may be acquired in this way. It is essential, however, that the mother be infected and in most instances this infection is localized in the placenta. Smallpox, scarlet fever, measles, dysentery, various pyogenic infections, and in rare instances pneumonia, have also been acquired by placental infection. In rare cases in animals, anthrax, symptomatic anthrax, chicken cholera, and glanders have been acquired by antenatal infection from the mother.

Variation in Infection

It should be noted that there may be a variation in the infection depending upon the route by which the infectious microörganism enters the body. For example, in the case of *Bact. tuberculosis*, if the bacterium enters the skin a usually non-fatal infection called lupus vulgaris results; if it enters the lymph glands or joints and localizes there an inflammation of not necessarily a fatal character results; if it enters the lungs pulmonary tuberculosis or consumption occurs which usually, after being well established, runs a fatal course; if it enters the intestine, intestinal tuberculosis may result which is nearly always fatal; and if it enters the meninges, tubercular meningitis results which is rapidly fatal. Just so with the *Strept. pyogenes*, depending on whether it enters the circulation, the lymphatic vessels of the skin or the connective tissues there results septicemia, erysipelas, or abscesses which obviously differ in their severity. The same is true of practically all the pathogenic bacteria which invade the plant and animal body, the variation in the route produces a great variation in the type and the results of the infection.

It was mentioned in the beginning of this discussion that *infection* included certain things such as the entrance of bacteria into the body tissues, their increase and their injury to the body. There is some variation in what constitutes an infection depending upon the infectious microörganism and the tissue it attacks. For example, *Msp. comma* of Asiatic cholera does not produce an infection unless it comes into contact with the intestinal mucosa and in this case it does not enter the tissues but sets up an inflammatory process on the surface. If this same bacterium comes into contact with tissues such as those of the nose, throat, lungs, no infection results. In the case of *B. typhosus* of typhoid fever the bacterium not only attacks the intestinal mucosa, but in addition it enters the tissue of the lymphatic patches and sets up an inflammation. This microörganism may also invade the circulatory system directly. In order for such an organism as the *Strept. pneumoniæ* to produce pneumonia it is only necessary for the bacteria to come into contact with the thin, single-celled, alveolar wall through the blood or air passages. In case this bacterium produces an abscess it is necessary for it to first enter into the tissues. In the pneumonic form of plague, although infection is supposed to be accomplished generally by the inhalation of bacilli, the *Bact. pestis* may be carried to the alveolus through the circulation and thus enters the tissues of the lung before actually invading the alveoli. This sometimes occurs in case of *Strept. pneumoniæ*. It also gives rise to abscesses occasionally but only when it invades lymphatic glands. The same is true of the large number of infectious microbic agents; there is a variation in the infection due to the variation in the microörganism and the point where this agent attacks the body. The severity of an infection, as for example, a pneumonia due to *Strept. pneumoniæ* or to *Strept. pyogenes*, or to *Bact. pneumoniæ* (Friedlander) or to *Bact. pestis*, would vary with the infectious agent, its virulence and number, and with the resistance of the individual infected.

The Factors which Influence the Results of an Infection

There are four principal factors which influence the results of an infection. They are as follows: The virulence of the infecting microorganism; the number of the infecting microörganisms; the avenue by which the infectious microörganism enters the body; and the resistance of the plant, animal or individual infected.

Virulence.—It is a self-evident fact that the more virulent a microorganism is the more serious will be the infection which results from its invasion of the body. There is a great difference in the virulence. For example, *Strept. pyogenes* may infect the skin of mucous membranes and produce only an abscess of varying proportions. Again, it may be more virulent. The resistance of the infected individual may be lowered somewhat and the streptococcus may enter the lymphatics of the skin and produce erysipelas or the blood stream and produce a fatal septicemia. Furthermore, one strain of the streptococci in the blood may produce a very virulent infection and another a less severe one. Virulent streptococci are not readily phagocytized by the leucocytes. The same variation in virulence is noted in all the pathogenic bacteria and the infections are modified thereby. The fact that an organism is virulent for an animal is not evidence that it is virulent for man. The virulence of an organism depends upon its ability to form toxins or other poisonous substances.

Number.—The number of infecting microörganisms which are introduced into an animal body is of importance. In anthrax, for example, it has been shown that one bacterium is capable of multiplying and setting up an infection. In tuberculosis and typhoid fever and most of the infectious diseases it requires a rather large number before an infection will take place. The leucocytes, bactericidal substances in the blood, and the body cells in general are capable of destroying many infectious agents. Furthermore, it can be readily understood how a few bacteria might be able to cause a mild infection and an increasing number be able to so overcome the bodily resistance as to cause a more or less severe infection.

Avenue.—It has been pointed out previously how the avenue of infection modifies the infection. A very virulent microörganism may occasionally produce a very mild infection when introduced in a certain locality while in another place the same organism may produce a very severe type. The results of the infection will be materially modified depending on the avenue of entrance which the virulent microorganism takes. For example, in addition to those mentioned previously, suppose *Bact. pestis*, the causal agent of plague, enters the blood through the skin, or the lymphatics through the tonsils, it is carried to the lungs and there produces a very severe and usually fatal pneumonia; if bacteria enter the lymphatic system in large numbers they frequently localize in the lymph glands producing buboes or glandular enlargements which are not always fatal. These bacteria may also enter the blood current and produce a rapidly fatal septicemia. It has not been established in man that plague can be produced by the ingestion of *Bact. pestis*, but in some susceptible animals such as rats, the disease in a very fatal form is rapidly acquired when the bacteria enter the intestines.

RESISTANCE.—This factor is one of the prominent ones which modify the results of an infection. It is a familiar fact that two or more individuals may be infected with the same microörganism, as for example, *B. typhosus*, and one will not become infected or have a very mild form of the disease, while the other will have the severest and most fatal form of typhoid fever known. Again, the age of the individual infected is important in determining the resistance. The adult resists infection such as diphtheria, scarlet fever, and measles more than the child. The very young child resists pneumonia and tuberculosis more than the adult. The resistance of the body depends on the presence in that body of natural or acquired antibodies. It is, therefore, obvious that the higher resistance or immunity of the individual infected, the less severe will be the results of the infection on that individual.

THE EXACT CAUSE OF INFECTIONS

We are familiar with the fact that all of our infectious diseases are due to microörganisms or viruses of some form or other. The causal agents of many of these diseases are known but in the case of those that are not known there is reasonable certainty as to the types of the infecting agents. The exact substances which are produced by the microorganisms and which are responsible for symptoms of the various diseases will be briefly considered in the following paragraphs.

SOLUBLE TOXINS.—It is a known fact that there are some pathogenic bacteria which secrete through their cell walls poisons which diffuse into the surrounding media. To these poisons or toxins the disease symptoms are due. *Bact. diphtheriæ* of diphtheria, *B. tetani* of lock-jaw or tetanus, *Bact. dysenteriæ* of bacillary dysentery, *B. botulinus* of meat poisoning, and *Ps. pyocyanea*, the causal organism of blue-green pus, are about the only bacteria of this character. Some bacteria, such as *Strept. pyogenes* and *M. pyogenes var. aureus* produce hemolytic toxins. There are certain protozoa as, for example, certain entamœbæ and the various trypanosomes which secrete soluble poisons. Among the animals, the venoms of the poisonous snakes, the poison of the centipedes and spiders, the serum of the eel, and the excretion of the dermal glands of the toad are examples of secreted toxins (zoötoxins). Again, among the plants abrin from the jequerity bean, ricin from the castor oil bean, and others, are examples of soluble toxins the product of plant cells (phytotoxins). The cells producing these toxic substances, therefore, are only indirectly responsible for the infections for it is the toxins themselves which produce the pathogenic effect on the body.

ENDOTOXINS.—Many of the pathogenic bacteria and some of the protozoa do not secrete their toxins outside the cell wall but hold them within the wall in combination with the protoplasm. They do not liberate these substances until the microörganisms die and are disintegrated. Such toxic substances are called *endotoxins* to distinguish them from those secreted from the cell, namely, the *soluble toxins*. Two of the best examples of pathogenic bacteria of this type are the *Msp. comma* of Asiatic cholera and *B. typhosus* of typhoid fever.

TOXIC BACTERIAL PROTEINS.—There are some bacteria and other parasitic cells which produce a small amount of endotoxin and in certain instances some soluble toxin but not enough of either of these substances to account for the toxicity of the organism. It has been found that when organisms of this character are ground up and washed to free them of their endotoxin and are washed free of all soluble toxins, they are still toxic. It has been shown that this toxicity is due to the protein substances of the cell. The *Bact. tuberculosis* and the *Bact. mallei* of glanders are two notable examples of microörganisms of this character. When, for example, the proteins of *Bact. tuberculosis* are injected into the circulation of susceptible animals, tubercle formation occurs showing that these proteins are poisonous.

Other Possible Exact Causes.—In certain infectious diseases it is also claimed by certain writers that enzymes are responsible. This lacks substantiation. It is also stated that in such infections as anthrax the mechanical effect of the bacteria plugging up the capillaries and producing mycotic emboli is a factor. This may be true but in addition other factors are concerned as previously mentioned.

In mixed infections of two or more organisms, which frequently occurs, the infected individual may have within the body soluble toxins, endotoxins, and toxic bacterial proteins and in such a case it is difficult to differentiate their action.

The Methods by which Infectious Microörganisms are Disseminated

The microörganisms of some of the infectious diseases such as diphtheria and Asiatic cholera and usually tetanus remain local and seldom enter the body generally. From the locus of the infection they disseminate their toxic or poisonous products. In the case of tetanus the toxin is carried over the body along the sheaths of the motor nerves; in diphtheria the toxin is usually carried by the lymph, occasionally by the blood; and in the case of cholera the blood and lymph both serve to carry the toxic agents. In diphtheria and cholera the microörganisms very frequently extend along the mucous membranes from the original point of infection. There are other infections in which the causal microörganisms extend only from the point of original invasion into the surrounding areas. Such is the case with *Strept. pyogenes* in the infection of the lymphatics of the skin in erysipelas and of *Bact. influenzæ* in all infection through the respiratory tract. Many infectious agents are carried by the blood and occasionally by the lymph, as for example, in tuberculosis, syphilis, glanders, plague, leprosy, pneumonia, and the septicemias due to the pyogenic cocci. It is possible in certain cases that the leucocytes acting as phagocytes may carry virulent infectious agents through the blood and lymph from one part of the body to the other.

The Methods by which Infectious Agents are Eliminated from the Body

The etiological microörganisms of the various infectious diseases may be eliminated from the body in two general ways, namely, by a direct method and by an indirect method. For a microörganism to be directly eliminated from the body it is necessary for the focus of the infection to communicate with the outside of the body in some way or other. In the case of infections of the mucous membranes and the skin there is, of course, direct communication with the outside. In diseases of the respiratory organs and the intestines the infectious agents are discharged into the lumen of the air passages and the intestines and then thrown out from these passages. Examples of the partial direct elimination from the skin may be found in such diseases as smallpox, measles, syphilis, scarlet fever, lupus vulgaris, and in suppurative conditions such as carbuncles and furuncles. From the present evidence little significance perhaps is to be attached to the elimination of the infectious agents mentioned directly from the skin. It is probable that the microorganisms which are eliminated remain alive for only a short time and are not factors of consequence in the transmission of these infections. As examples of diseases in which direct elimination from the various mucous membranes occurs, infections such as typhoid fever, tuberculosis, cholera and dysentery from the membranes of the intestines; influenza, pneumonia and tuberculosis from the bronchial membranes; diphtheria, leprosy, glanders and scarlet fever from the membranes of the nose, throat, and tonsils; and gonorrhea, syphilis and tuberculosis from the membranes of the genito-urinary tract may be mentioned. In elimination from the various internal membranes sometimes reinfections occur such as in the case of the elimination of *Bact. tuberculosis* from the respiratory tract, the swallowing of the sputum, and the subsequent infection of the intestines.

In the second, or indirect method of elimination, two distinct propositions present themselves; first, the infectious microörganism must enter the lymphatic or blood circulation; and, secondly, in order to get out of the body they must pass through the cells of some of the organs, the mucous membranes or skin. It is a common occurrence for bacteria and other microörganisms to get into the circulation in some of the infectious diseases such as typhoid fever, pneumonia, plague, and in the various septicemias. They may pass through the epithelium of the kidney and be eliminated in the urine; they may pass through the liver and be eliminated in the bile, finally passing out through the intestines; and they may pass through the mucous membranes of the intestine and possibly pass through the glandular epithelium of the sebaceous and sweat glands and be eliminated through the skin. They have also been known to pass through the glandular epithelium of the milk glands when these glands are not grossly diseased and through the salivary glands. It has been recently well demonstrated that there must be some form of lesion in the liver and kidney in order for the microörganisms to pass through. Infectious microörganisms are sometimes destroyed by the lysins in the blood, carried to and deposited in the spleen and bone marrow and gradually disintegrated and dissolved.

In certain infections in which a recovery seems to have occurred all the infectious microörganisms are not always eliminated from the body. As mentioned previously, *B. typhosus* and *Bact. diphtheriæ* are frequently carried by persons fully recovered from these diseases. Sometimes, however, inflammatory infections are set up by these bacteria. It has been suggested on seemingly good evidence that inflammations of the gall-bladder and gall-stone formation may be due to the toxic action of the bacteria of typhoid fever which have been retained in the gall-bladder for a considerable time following an attack of typhoid fever. It is known that frequently repeated attacks of malaria are due to the retention of some of these protozoan parasites for a time in the quiescent stage. Repeated attacks of erysipelas caused by the *Strept. pyogenes* may also be due to the same condition. It is also claimed by some (Von Behring) that *Bact. tuberculosis* is taken into the body in infancy, that it is not eliminated, and that it sets up infection in later life.

In conclusion should be mentioned one other indirect way in which infectious agents are eliminated from the body, namely, by being taken up by suctorial insects from the blood. It is necessary that this be done in order to perpetuate the parasite and complete its life cycle in certain instances, as with the mosquitoes in yellow fever and malaria. In other instances the parasites are only taken up by the insect and subsequently injected into another individual or digested as the case may be. This occurs with the ticks in the transmission of certain of the piroplasmoses and with the tsetse flies in the transmission of certain of the trypanosomiases.

THE EFFECT OF INFECTIOUS MICROÖRGANISMS ON THE BODY

It becomes necessary to consider briefly the effect of the various infectious microörganisms and their toxic substances on the body.

THE PERIOD OF INCUBATION.—This period is that elapsing after the entrance of the infecting organism into the body until the symptoms of the disease develop. This period is variable in most diseases and depends upon the same factors which modify the results of an infection, namely, the virulence of the infecting organism, the number growing in the body or its tissues, the avenue, and the resistance of the individual. The period can be in a measure controlled and shortened in experimental animals by inoculations into the circulatory system and in other regions depending on the organisms used. In some of the human diseases, particularly those of unknown cause, the period of incubation is quite constant, as for example, in smallpox and measles.

LOCAL REACTIONS.—The local effects of the toxic substances of microörganisms are usually first inflammatory and later possibly necrotic, that is, they produce a death of the tissue involved. The inflammatory changes may be confined to those of an acute character as, for example, in the various serous, hemorrhagic, suppurative and fibrinous inflammations, or be chronic and proliferative in nature. There is always a variation in the type of inflammation depending on the location of the infection and also a variation in two different individuals of the same species infected at the same point with the same agent. In some diseases such as tetanus the local point of infection may entirely heal and still the bacteria be localized at this point and disseminate their toxin. In some cases of tuberculosis and glanders the bacteria may become localized at the points of infection and after an acute inflammatory stage the point may become the seat of a chronic process and proliferative changes occur in the tissues.

GENERAL REACTIONS.—*Metabolism.*—The general metabolism of the body is affected by the changes produced in the amount and the chemical constitution of the food substances which are taken into the body. By changing in the same way the substances which naturally are eliminated from the body and by setting up new and abnormal changes in the functional activity of the tissues, the general metabolism may be disturbed. Muscular weakness, delirium, pain and loss of appetite, together with vomiting, diarrhea, disturbance of intestinal absorption and the digestive juices are often noted in cases of altered metabolism. The fats, carbohydrates, and then the proteins are in the order named rapidly used up, producing certain changes in the respired air and in the urine and feces. Infectious microörganisms may also reduce the power of the hemoglobin to carry oxygen and perhaps cause a narrowing of the respiratory passages thus preventing the necessary amount of oxygen reaching the lungs and subsequently the tissues.

Infecting microörganisms may alter the composition of the food substances and after being taken into the body produce abnormal compounds which have little or no nutritive value on absorption or they may produce toxic substances related to ptomains. Substances which normally should be eliminated from the body are often retained and abnormal losses of such substances, as water and various albuminous compounds, occur.

Attendant upon the changes in metabolism usually there occurs *fever* in all infectious diseases. It is probable that the fever is the result of the effect of the toxic protein compounds of the infecting microörganisms on the tissues, or the disintegration of the protein compounds of the body due to the action of toxins. It is evident that the fever-producing substances, in certain infectious diseases, act in a very characteristic manner as is demonstrated by the so-called typical fever curves. It seems to have been demonstrated that fever is a good sign and that it is indicative of the reaction of the body to the toxic substances of the infecting agents. It has also been shown that the fall of fever in certain infections is attendant upon the formation and saturation of the body fluids with antibodies.

Blood-forming Organs.—There are usually changes in the blood-forming organs in most all types of infection. The spleen frequently shows enlargement and contains numbers of myelocytes which are also found in the blood in increased numbers. This is probably due to the disintegration and deposition there of red blood corpuscles and also to the action of toxic substances. The endothelial cells and leucocytes of the spleen are actively phagocytic. The bone marrow, particularly the fatty marrow, shows large numbers of myelocytes of the neutrophile type and it eventually becomes lymphoid in nature in a large number of infections. The lymph glands also frequently show endothelial proliferation.

Parenchymatous Tissues.—All sorts of degenerations of the kidneys, heart, liver and some of the other organs frequently occur in infections. Amyloid formation and necrosis of tissue sometimes occurs. Many of the toxins have special affinities for tissues, such as the tetanus toxin for nerve tissue and this produces organic changes. It is possible also that the fever is responsible for a certain portion of the changes in the parenchymatous organs in infections.

Epithelial and Endothelial Tissues.—In certain infections, as for example, diphtheria, the epithelial tissues are subject to inflammation; and in other infections, as for example, syphilis, the endothelial tissues of the blood-vessels undergo inflammation and sometimes proliferation. The epithelial and endothelial cells are frequently actively phagocytic and large numbers of the infecting microörganisms are taken up and destroyed. Some of the infectious microörganisms produce no effect whatever on these tissues, while others produce pronounced destructive changes.

Erythrocytes and Leucocytes.—Lytic or dissolving substances for the red blood corpuscles are frequently produced in infections (hemolysins). *Strept. pyogenes, M. pyogenes var. aureus*, and *Ps. pyocyanea* are among the bacteria which produce hemolysins. Anemia is, therefore, not an uncommon finding in many infections. Normal human blood and that of some animals contains an antilysin for the staphylolysin and it is sometimes produced in large amounts. Agglutinating substances for red corpuscles are produced by some pathogenic microorganisms and it is possible that these are the cause of the so-called agglutination thrombi which occur in infections like typhoid fever.

The most marked changes seen in the leucocytes in infections is their rather constant increase in number; in most cases a leucocytosis. In uncomplicated tuberculosis and typhoid fever, in measles and German measles, in malaria, and in dengue, there is no increase in number. In acute inflammations it is the polymorphonuclear leucocytes that undergo an increase. This increase is sometimes preceded by a decrease (leucopenia). The leucocytes act as the principal phagocytes of the body and are attracted (positive chemotaxis) to the bacteria or other microörganisms after they have been sensitized by the opsonins in the body fluids. Besides acting as phagocytes they may, according to Metchnikoff, produce antitoxins and bactericidal substances. It has been suggested that the initial leucopenia in some cases is due to the production of negative chemotactic substances. Some virulent bacteria cannot be phagocytized probably because they produce very strong negative chemotactic substances.

Antibody Formation.—One of the general results of pathogenic microorganisms in certain infections is the production of antibodies of various kinds. These may be antitoxins as in the case of tetanus and diphtheria, or bactericidal substances as in typhoid fever and cholera, or opsonic substances as in the pyogenic infections. Agglutinins, precipitins, and other bodies are also sometimes produced in conjunction with the other immune substances mentioned.

CHAPTER II*

IMMUNITY AND SUSCEPTIBILITY

GENERAL

DEFINITION.—A clear understanding as to what is meant by the terms immunity and susceptibility is of fundamental importance. By *immunity* we mean the resistance which an animal or plant body possesses to the etiological microörganisms of an infectious disease and to the disease itself. The name has been adapted from the Latin *immunis* which meant a person who was free or exempt from public duties and later, one who was exempt from the action of poisons. Briefly stated, *immunity is resistance to disease.* It results commonly as a natural termination of the process of self-healing in many infectious diseases. The absence of such resistance, which may be total or partial, characterizes what is known as *susceptibility.* Throughout the animal kingdom and also among the plants there is a great variation in the immunity and susceptibility in the different species to the various diseases. Immunity bears no relation to the contagiousness of a disease and the term is only applied as a rule to strictly infectious diseases and not to metabolic diseases.

HYPERSUSCEPTIBILITY OR ANAPHYLAXIS.—It has been shown that animals and man are occasionally hypersusceptible to certain proteins. For example, there are individuals who are always seriously poisoned by the ingestion of eggs, pineapples, and strawberries. Certain individuals when injected with diphtheria or tetanus antitoxin which is carried in horse serum are seriously intoxicated and occasionally die. In such instances the proteins of the horse serum and not the antitoxin are responsible. It has been demonstrated that an animal may be sensitized or rendered hypersusceptible to almost any protein by first injecting a minute amount of the protein and then, after a period of at least eight to thirteen days, may be seriously intoxicated, if not killed, by the injection of a slightly increasing dose of the same protein. The proteins of the bacterial cells have been shown to act in the same way. Animals injected, as described above, may be rendered hypersusceptible to all bacterial proteins. Furthermore, as referred to above, individuals may be naturally hypersusceptible to bacterial and other proteins. The manner of the original sensitization in these cases is not known. Sensitization which has been either naturally or artificially acquired is transferred in most instances *in utero* to the first generation; that is, a mother may be sensitized, convey the sensitizing substances to her young while in the uterus, and when these offsprings are subsequently injected after birth with the same protein they may be intoxicated or killed. In this connection it should be stated that the so-called inherited tendency to specific diseases may be something more definite than we are ordinarily accustomed to regard it. Suppose a mother becomes tuberculous and is, therefore, sensitized to the proteins of *Bact. tuberculosis;* it is quite possible for her to convey to the offspring this susceptibility to the particular proteins of *Bact. tuberculosis* as has been demonstrated artificially. After birth, or in later life, when the causal microörganisms of tuberculosis are taken into the body, as they are in about ninety-five per cent of all persons in civilized countries, the bacteria may find a more than ordinarily susceptible individual and may develop with comparatively little hindrance. If this condition be true naturally as it is when produced experimentally in susceptible animals a very interesting and scientific explanation of the so-called inherited tendency to tuberculosis is at hand.

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* Prepared by E. F. McCampbell.

PREDISPOSITION AND NON-INHERITANCE OF INFECTIOUS DISEASES. There is probably no such thing as a truly inherited infectious disease. This point has been debated and discussed for a great many years and the above conclusion has been reached by the majority of investigators. By *inheritance* is meant the transference of a property, or in this instance, a pathogenic microörganism by the nuclear substance of either the spermatozoön or the ovum. It is only the nuclear substances which combine to form the new individual. It is true among certain of the lower animals, such as the fowls and some insects, that microörganisms are carried within the egg but the eggs are quite different in structure from the human or mammalian ovum. The egg of the above-mentioned animals is composed largely of yolk-furnishing food and there is ample opportunity for microbic growth, while the mammalian ovum contains no yolk. Such instances should be referred to as *germ-cell transmission*, not inheritance. If the microörganisms were present they would be immediately incorporated within the new developing embryo. If the microörganism ever did find its way into the human or mammalian germ cells it would be a mechanical impossibility for the cells of the embryo to divide and multiply in proper manner. Such pathogens would rapidly destroy the developing cells in the embryo. It is true that the offspring of certain individuals are born diseased. For example, children are not infrequently born with syphilis and tuberculosis. At first thought it might seem that this is inheritance but on careful analysis it will be found that the mother is either syphilitic or tuberculous. Furthermore, the focus of the infection is most frequently in the uterus and the microörganisms are transferred to the unborn offspring by means of the fetal circulation. This condition is what is known as *antenatal acquirement;* it is not heredity. It is absolutely impossible for the male to communicate any disease to the offspring unless the female is first infected. Colle years ago formulated a law which bears his name in which he stated that a father could communicate syphilis to his child without the mother being infected. This law has been disproved since the introduction of the new serum tests for syphilis and it can be positively demonstrated in all such cases that the mother is infected. Ante- or prenatal acquirement may then be recognized. What can be said in regard to the predisposition to a definite infectious disease? There is a question as to whether true predisposition does exist. Many cases are on record to show that disease seems to run in families and in localities. For example, tuberculosis and cancer are frequently said to be subject to inheritance or to predisposition in certain cases. It can be easily seen that if one parent is diseased the germ cell of the parent will be less healthy and when combined with a normal healthy germ cell of the other parent will not give rise to as healthy an individual as when both cells are from healthy individuals. Again, the result when the germ cells of both parents are unhealthy, due to the parents being unhealthy, is evident. Predisposition seems to resolve itself into the inheritance of a weakened constitution, a constitution which will not withstand the ordinary infections easily. It seems not to be a predisposition to any particular disease but a predisposition to all diseases, infectious and metabolic. Diseases such as tuberculosis are so prevalent that it is very possible that infection may take place and it be interpreted as inherited because the parent died of the same cause.

As mentioned above, it may be that the true explanation of the phenomena of predisposition is found in anaphylaxis or the sensitization to various proteins of microörganisms. Further work is necessary along these lines.

IMMUNITY

Immunity and susceptibility to disease are always relative and never absolute; that is, it is always possible to produce some sort of an infection in a supposedly immune animal by modifying the conditions under which the animal is accustomed to live. For example, the chicken is immune to tetanus but by keeping this animal for some time at a temperature higher than its normal it may be infected. The cow cannot ordinarily be infected with typhoid but when large numbers of the *B. typhosus* are injected under the skin an abscess may be produced. These and many other examples might be mentioned. Our standard of immunity in a particular animal is based upon the conditions as they exist naturally and on the average resistance of animals of the same species.

Immunity to disease may be of two kinds, natural and acquired. *Natural immunity* is that resistance which is possessed normally by an individual. *Acquired immunity* is that resistance which is acquired by having an infection, or by being vaccinated, or immunized against an infection with the specific etiological microörganism or its antiserum.

NATURAL IMMUNITY AND SUSCEPTIBILITY.—Attention should be directed to certain forms of natural immunity and susceptibility.

Racial Immunity and Susceptibility.—It is a familiar fact that certain species of animals and certain races of man differ in their resistance and their susceptibility to infectious diseases. As examples of racial immunity among animals the native cattle of Austria and Hungary and of Japan which are relatively immune to bovine tuberculosis, a disease which causes great loss among other breeds, may be mentioned. Again, the sheep of Algeria are relatively immune to anthrax while all other sheep are extremely susceptible. Field mice are immune to glanders while the common house mouse is susceptible. The negro is more resistant to the infectious microbic agent of yellow fever than other races, but is without doubt more susceptible to tuberculosis. The Japanese are said to be more resistant to scarlet fever than other races. The

Melanesians are very susceptible to measles and the Malaysians to beriberi, while other races are relatively immune.

Familial Immunity and Susceptibility.—It is true that certain families vary in their immunity and susceptibility when compared with other families in the same community. For example, tuberculosis undoubtedly shows a tendency to run in families. In determining a case of this kind it is, of course, necessary to take cognizance of the environment of the individual and the association with other diseased persons. The so-called tuberculous diathesis does exist and perhaps we have an explanation of it in anaphylactic phenomena as mentioned previously. Measles and scarlet fever also in certain instances seem to run in families.

Individual Immunity and Susceptibility.—Variation among individuals associated together is noted in regard to their resistance and susceptibility to disease. It is well known, for example, that in a herd of cattle, which are in the main tuberculous, there are certain individuals who never contract the disease. These animals may be of the same breed and be fed and handled the same as the rest of the herd, still they never become infected. Again, in the human race, with the acute exanthematous diseases such as scarlet fever and measles, there are children, for example, in the same family and of nearly the same age and living under exactly the same conditions, who contract the disease and others who do not. The exact cause of the individual, familial and racial immunity cannot be satisfactorily explained at the present time. There is also a variation in the individual's resistance at different times dependent upon food, sleep, work and general hygienic conditions.

FACTORS OF NATURAL IMMUNITY.—The natural immunity of any individual to an infection may be dependent upon several things as follows:

The Protection Afforded the Body by the Surfaces.—The body surfaces may be conveniently divided into those which are external and those which are internal.

Skin and Cutaneous Orifices.—The first protective mechanism that we wish to call attention to is the skin. It is a well-known fact that virulent bacteria are frequently present on the skin of seemingly normal and healthy individuals. Perhaps the most common of these is the *Strept. pyogenes* and the *M. pyogenes vars. aureus* and *albus.* These microörganisms and others live largely as saprophytes, feeding upon the dead and desquamating epithelial cells. The skin is impermeable to these microörganisms when it is unbroken in its normal state. Experiments have been performed to determine whether the skin is normally permeable to bacteria. Bacteria have been rubbed into the skin and have produced infection but in these instances the skin has been abraded by the mechanical irritation. Bacteria may infect the sudoriferous and sebaceous glands and their ducts, in case the metabolic activity of these structures is disturbed. The ducts and the glands of the skin are protected ordinarily by a flow of the secretions. In case the flow of the secretions is decreased and the orifices of the ducts contracted as in cold weather, while bacteria find it more difficult to pass down than before, they occasionally do produce an infection. When a hair follicle is diseased and the shaft contracted or perhaps dropped out, bacteria may pass down and produce an infection. *B. tetani* of tetanus or lockjaw frequently passes through the skin by means of deep penetrating wounds. The same is true of some other pathogens.

In case bacteria are successful in permeating the skin either directly or by means of cutaneous orifices, they are usually able to set up a marked inflammation of these structures and produce necrosis of the epithelium. It is in this way that pustules, boils, carbuncles, and various forms of cellulitis are produced. The secretions of the sebaceous glands are not germicidal but are perhaps slightly antiseptic due to the salts which are contained therein. Furthermore, as soon as the serum from the blood is extravasated there may be slight germicidal action on the bacteria infecting the skin. The soluble toxins of bacteria cannot be absorbed through the unbroken skin.

The Subcutaneous Tissue.—In case the bacteria are successful in permeating the skin and penetrating the subcutaneous connective tissue, again various protective mechanisms show themselves. This resistance is due to a very rapid production of new connective tissue which serves to mechanically limit the infection. It is due, furthermore, to the germicidal action of the serum, the mechanical and germicidal action of the fibrin and the phagocytic activity of the leucocytes. These various factors will be discussed subsequently in connection with the phenomena and the protective mechanisms of inflammation.

The Exposed Mucous Membranes of the Body.—The exposed mucous membranes of the body usually are covered with a variety of bacteria, some of which are pathogenic. Their moist condition favors the growth of microörganisms, but the mucus which is secreted upon them forms a mechanical barrier to the bacteria and serves to wash them away. This mucus is not germicidal but is perhaps slightly antiseptic. The only mucous membranes of the body that are really exposed are those of the eyelids, lips, anterior nares, genito-urinary apparatus and the anus. It is perhaps more convenient to discuss these membranes in detail in connection with the cavities which are connected with them.

Nasal Cavity.—Microörganisms find a barrier to the entrance of the nasal cavity in the hairs which protect the anterior nares and serve to keep out the dust from the inhaled air. The membranes of the nasal tract, besides being covered with mucus, which acts as above mentioned, are also covered with ciliated epithelial cells which move from within out and serve to wash the mucus containing the bacteria from the surface. Infections of the nasal mucous membranes are, however, not uncommon. *Bact. influenzæ*, *Strept. pyogenes*, *M. pyogenes vars. aureus et albus*, *Bact. diphtheriæ*, *M. intracellularis var. meningitidis*, and occasionally *Bact. mallei* produce infection through this membrane.

The Mouth.—The mouth probably contains the largest variety of bacteria to be found anywhere in the body. A large number of these bacteria are non-pathogenic, although pathogenic microörganisms do occasionally occur. All the requisite conditions for bacterial growth are provided in the mouth, namely, temperature, moisture and food. The food supply is largely derived from materials which have been deposited during the process of mastication between the teeth and in the various depressions of the mucous membrane. The microörganisms also feed upon the desquamated squamous epithelial cells. They are being constantly washed off the membrane by the saliva which contains a certain portion of mucus. The saliva is not germicidal, and in all probability only very slightly antiseptic. The most permeable part of the mouth is in all probability the tonsils which separate this cavity from the pharynx or throat. These lymphatic structures have many deep crypts, and bacteria once entering the tissues of the tonsils may gain access to the lymphatic circulation through these structures.

In case bacteria are successful in passing the obstacles of protection afforded in the nose and in the mouth and pass into the throat, there are two routes for their entrance into the internal body, namely, through the trachea and bronchi into the lungs and through the œsophagus into the stomach and intestines.

The Lungs.—In case infectious microörganisms pass down the trachea and bronchi they meet first with the obstruction of the mucus which is secreted upon the surfaces of these tubes. In addition, ciliated epithelial cells are present and serve to cleanse the surfaces from microörganisms as in the nose. Occasionally microörganisms lodge along the trachea and the bronchi and produce slight irritations which if left undisturbed may immediately produce serious infections. However, the leucocytes from the neighboring bronchial and mediastinal lymph glands pass through the walls of the trachea and bronchi, ingest the microörganisms, carry them back to the glands and in a majority of instances destroy them. Occasionally, however, leucocytes containing virulent microörganisms get into the lymphatic circulation and these are carried by the diffusion currents in the lymph vessels down to the alveoli of the lungs and here may cause inflammations of a more or less serious character. It is probable that the *Strept. pneumoniæ* is very frequently carried to the alveoli of the lungs in this way. Without doubt, microörganisms cannot be directly inhaled through the air passages into the alveoli of the lungs during an ordinary inspiration, but it has been shown that in forced inspirations, such as those attending upon coughing, hiccoughing, sneezing and sighing that they may be so carried.

The Stomach.—In case the microörganisms pass down the œsophagus into the stomach, they immediately come into contact in the normal organ, with the gastric juice, which contains the hydrochloric acid in such concentration that it is at least antiseptic if not germicidal. In case the functional activity of the stomach is disturbed and the hydrochloric acid is diminished in amount, microörganisms may grow in the stomach to a limited extent. Furthermore, in case all the particles of food are not thoroughly broken up in the stomach, bacteria which may be contained within these particles may pass through the stomach into the intestine.

The Intestines.—In the intestines the microörganisms come into contact with the alkaline pancreatic juice which is slightly antiseptic and with the bile which is antiseptic and in certain instances bactericidal. They find no particularly favorable conditions for growth in the upper part of the small intestines under normal conditions. Here also mucus covers the surfaces. However, if the functional activity of the small intestines is disturbed, bacteria may enter the lymphatic structures (Peyer's patches, solitary follicles) low down in the small intestines and produce infection. Such is the case with *B. typhosus* of typhoid fever and with the *Msp. comma* of Asiatic cholera. Bacteria which have been prevented from development in the small intestines frequently find the opportunity in the large intestine. Here the concentration of the various digestive juices is lowered and the requisite condition for maximum bacterial growth is provided. Nevertheless, infections of the large intestine with bacteria are not common but may occur, colitis of various forms resulting. The various dysentery amœbæ very frequently develop in the large intestine.

The Genito-urinary Tract.—The mucous membranes of the genito-urinary tract, varying in male and female, present the same features as those of other mucous membranes. Besides the secretion of mucus, various other acid-containing secretions are often present. In addition, in the urinary tract the mechanical factor of irrigation removes the microörganisms. Not infrequently, however, microörganisms do enter these mucous membranes and produce serious infections, such as the *Treponema pallidum* of syphilis, the *M. gonorrhœæ* and the *B. chancroidæ mollis.* Sometimes these membranes are infected with ordinary pyogenic bacteria.

The Conjunctiva.—The conjunctiva is protected against infection in several ways. First, the eyebrows with their hairs and the eyelashes prevent microörganisms and particles of dust and dirt carrying microörganisms from entering the eye. Again, the lacrymal secretion or the tears flowing across the eye from the outside in serve to wash this membrane. Bacteria are frequently washed off the conjunctiva and pass down through the lacrymal duct into the nose where they meet the obstructions which have been previously discussed. In all probability the tears are only slightly antiseptic and not germicidal at all. The conjunctiva is sometimes infected with microörganisms and furthermore serves as a point of entrance into the body when it itself is not infected. There is no doubt that the *Bact. influenzæ*, the *Strept. pneumoniæ*, and other microörganisms may enter the body and get into the lymphatic and blood circulation in this way.

It is evident, therefore, that the protection afforded an individual by the body surfaces is a decided factor in the natural immunity of that individual.

The Protective Nature of Inflammatory Processes.—It has been mentioned in a previous discussion that when bacteria successfully enter a tissue and develop in that tissue a complex local change results which is designated as *inflammation.* In the majority of instances inflammation is of a beneficial nature. Fundamentally, it is always beneficial. Few examples of the pernicious results from inflammation can be given. In this connection may be mentioned the thickening of the cerebral blood-vessels in syphilis and the increase of connective tissue in cirrhosis of the liver. In these instances the inflammatory processes are brought about by the reaction of the various tissues to the irritation of the infecting microörganisms. Unluckily these reactions are not on the whole beneficial to the body, but, as before stated, inflammation is usually beneficial and may be characterized as *the reaction of tissues to injury.* The exact processes of inflammation may be traced in case an infecting microörganism succeeds in entering the tissues of the body. The organism having produced its toxic substance first causes a congestion of the blood-vessels in the region (hyperemia). Following this localized congestion there is an extravasation of plasma from the blood-vessels. This plasma immediately on leaving the vessels coagulates or clots, producing throughout the infected area fibrin and blood serum. This fibrin serves in a mechanical way to limit the infection, and it has been recently demonstrated that the fibrin possesses germicidal properties in addition. Furthermore, the serum in a large number of instances exerts a bactericidal effect upon the microörganisms. Following the extravasation of blood plasma from the capillaries, the leucocytes pass out and gather around the infected area. These leucocytes are attracted to the area due to the presence of various chemical substances (chemotaxis). They will come as close to the microörganisms as possible, depending upon the effect of the toxins which have been produced. In certain instances they will ingest the bacteria and destroy them. In such cases, the bacteria having been removed, the inflammation rapidly subsides and the infection is, therefore, checked. Such are the characteristics of an acute inflammation. Inflammations, however, not infrequently may become chronic, depending especially upon the microörganism producing the infection, and in such a case the inflammation after passing through the acute stage, as indicated above, stimulates a proliferation of the connective tissue in the part infected. In such cases, around the outside of the ring of leucocytes, which have been unable to ingest the bacteria, young embryonic connective-tissue cells which are known as *round cells* are found. In case the inflammation progresses, the leucocytes are destroyed and the round cells next to the infected area assume more of an elliptical shape and are known as *epithelioid cells.* On the outside of this layer of epithelioid cells will be found newly produced round cells, and on the outside of the round cells an area of recently migrated leucocytes, those passing out in the beginning having been destroyed by the toxic action of the infecting microörganisms. Frequently the newly produced connective tissue passes on to the adult type and in this instance completely walls off the area of infection and the infecting microorganisms. In such cases the inflammation and the infection are checked. Among the diseases caused by microörganisms which have a tendency to produce chronic inflammation may be mentioned those of tuberculosis, leprosy, syphilis, actinomycosis and glanders. It is not an uncommon observation in man to note in the lungs and in other parts of the body healed areas of tubercular infection; areas that have been completely walled off by the development of adult fibrous tissue. It is probable that about ninety-five per cent of all individuals living in civilized communities are infected with *Bact. tuberculosis* some time during their lives. The inflammation produced by this microörganism passes through the acute stage and into the chronic before being successfully combated and thoroughly walled off. Such an area is known as a tubercle, and in the other diseases mentioned, similar areas of like structure are produced. It depends entirely upon the virulence of the infecting microörganisms and the resistance of the connective tissue of the individual infected as to whether healing will result.

Natural Antitoxins.—It is an observed fact that certain animals resist the action of toxins produced by bacterial and other plant and animal cells. The question arises as to whether these animals are immune to the toxins on account of the presence in their bodies of natural antitoxins or other substances. If antitoxin is present, it can be detected by experiments made by drawing off the blood serum of the animal and combining it in varying proportions with the toxin in question. These experiments may be made *in vitro*. When toxins and antitoxins are combined in proper proportion and incubated together a non-toxic molecule is produced which when injected into a susceptible animal will produce no effect. It is, of course, necessary in this connection to inject the animal with a minimum lethal dose of the toxin in question as a control. If no natural antitoxins are present in the serum of the animal in question, the animal experimentally injected with the combined toxin and serum will die as a result of the non-combination of the toxin. In this way natural antitoxins may be tested. Natural antitoxins for diphtheria have been detected in the blood serum of about fifty per cent of normal humans and in about thirty per cent of horses. However, their occurrence in other animals for this specific bacterium and for other species is comparatively rare, and the explanation of the fact that certain animals are immune to toxins must be found elsewhere. It has been shown for example that the frog is immune to tetanus toxin, and that, when this animal is injected with this toxin, a large part of the toxin remains unchanged in the circulation for a variable period of time and may be later drawn off in the blood serum, producing a toxic effect when injected into a susceptible animal. There are no natural antitoxins present in the blood serum of the frog and it has been found that the immunity of this animal is due to the fact that there are no cells in the body possessing the necessary side-chains (open valencies) for chemical combination with the toxin and the subsequent intoxication of the cells does not result. It seems that the best explanation of the fact that certain animals are immune to toxins is found in the fact that there are no chemical substances in the cells with which toxin can combine. It is probably not true that natural antitoxins explain all the phenomena in this connection.

Natural Antibacterial Substances.—Natural antibacterial substances are present in the blood serum and body fluids of a large number of animals. In order to demonstrate the presence of the natural antibacterial substances it is necessary to inject the experimental animal with a carefully washed culture of the bacteria in question. If the animal remains uninfected, two possibilities present themselves: First, the presence of natural antitoxins; and second, the presence of antibacterial substances. It is necessary, of course, to have excluded the possibility of natural antitoxins, it having been demonstrated that the organism injected produces its diseased effects by endotoxins held within the bacterial cell rather than by toxins. There is no evidence indicating the presence of natural antiendotoxins in any animal. The antibacterial action of the blood may be due to two constituents, namely, cellular substances (leucocytes) and chemical substances in the serum. The rat and the dog are both immune to anthrax but the immunity of the dog is not due to antibacterial substances but to the phagocytic activity of the leucocytes, while in the rat the immunity is not due to the leucocytes but to the antibacterial substances. In order to demonstrate the fact that the leucocytes are not responsible for the immunity in the given animal it is necessary to combine the bacteria in question with the leucocytes and serum *in vitro* and after incubation make a careful examination with these cells to see if they have taken up any bacteria.

Antibacterial action is due to two substances in the serum: First, the thermostable substance which combines with the bacteria called an *amboceptor;* and second, a thermolabile substance called a *complement,* which combines with the amboceptor after this substance has combined with the bacterial cell. It is sufficient to say at this time that these substances occur in normal sera and that the result of their combination with the bacterial cell causes the death of the bacteria and in some cases a lysis (solution) of the bacteria in addition.

There may be present in the blood of animals antibacterial substances of three kinds: First, those just killing bacteria (bactericidal); second, those killing the bacteria and dissolving them (bacteriolytic); and third, the leucocytes which are active in the ingestion of the specific microörganisms and subsequently digest and destroy them. In all probability the overactivity of leucocytes in every case of natural phagocytic immunity is due to the presence of normal opsonins—substances which sensitize the bacteria and render them susceptible to phagocytosis.

Normal Hemolysins.—Normal hemolysins (hemoglobin-liberating substances) are present in the sera of certain animals for the red blood corpuscles of other animals of different species, and for the same species, but never for the red corpuscles of the animal from which the serum was obtained. Such substances known respectively as *heterolysins* and *isolysins* and if the latter occurred the name *autolysin* would be applied.

Normal Agglutinins.—Normal agglutinins for various bacteria, such as *B. typhosus*, *Msp. comma*, *Bact. dysenteriæ*, *B. coli*, and *Ps. pyocyanea* are present in the blood serum of some animals. It is necessary, of course, to exclude normal agglutinins when testing the serum of the infected case for the purposes of diagnosis as will be mentioned later.

Normal Precipitins.—No normal precipitins for bacteria occur in the sera of animals. Precipitins for various blood sera, however, do occur. For example, human serum will precipitate the serum of certain species of monkeys. These substances will be discussed in detail under acquired immunity.

ACQUIRED IMMUNITY.—Acquired immunity is that resistance which is acquired after having an infection or from artificial inoculation with the etiological microörganism of an infection or from inoculation with the products remaining in the body after infection, whether natural or artificial. Acquired immunity may be divided into two classes, namely, *active* and *passive*. *Active immunity* is that immunity resulting from an infection or vaccination. In it the body cells react and give rise to the formation of antibodies. When antibodies produced in active immunity are inoculated into other animals the immunity conferred is referred to as *passive immunity*.

Active Immunity.—Active immunity may be produced artificially in the following ways: By the injection of living bacteria; by the injection of bacteria of reduced virulence; by the injection of dead bacteria; by the injection of the secretory and excretory products of bacteria (toxins, etc.); by the injection of the disintegration products of bacteria liberated after the death of the cells (endotoxins); and by the injection of bacteria or bacterial products which in no way are related to the bacterium against which immunity is conferred.

As a result of the injection of living bacteria in small amounts or of bacteria of reduced virulence the body cells react and produce bactericidal substances (lysins, etc.). As a result of the injection of dead bacteria, the opsonins are increased in the blood. As a result of the injection of the secretory and excretory products of the bacteria, namely, toxins, antitoxins are produced. As a result of the injection of the disintegration products of bacteria, namely, endotoxins, bactericidal substances are produced. In cases where bacteria or bacterial products, which are in no way related to the bacterium against which immunity is conferred are injected, it is probable that bactericidal substances are produced. This condition only occurs in rare instances.

Passive Immunity.—Passive immunity may be conferred by the injection of antitoxins, and by the injection of bactericidal substances. In this type of artificially produced acquired immunity the body cells do not react to any great extent and the injected antibodies remain practically unaltered. Various other antibodies may be injected into other animals and confer upon them passive immunity.

The principal antibodies produced in active immunity will be subsequently discussed.

THE ORIGIN AND OCCURRENCE OF ANTIBODIES

The toxic and some of the non-toxic substances of bacteria and cells from other sources when introduced into the body of a susceptible animal usually have the power to produce *antibodies*. Substances having the power of producing antibodies are known as *antigens*. Among the antibodies produced are antitoxins, bactericidal and lytic substances, opsonins, antiferments, agglutinins and precipitins. The antigenic substances for these antibodies will be discussed later. The mechanism of action of the antigen is of interest. It is supposed that the antigen can combine only with the cell which has the proper combining groups or receptors. The antigen combines in the same way that food products combine with the tissue cells. In case there is no group in the tissue cell with which the antigen can combine that tissue is naturally immune to the antigenous substances in question. In the same way tissue cells cannot utilize certain foods because they have no combining groups. If all the tissue cells in the body are in this condition then the individual may be said to be naturally immune. It occasionally occurs that certain cells of the body are not susceptible to the action of antigens at one time while at another they are susceptible. For example, the red blood corpuscles of the young chick are not affected by the lysin-toxin in spider poison while those of the adult are readily hemolyzed (hemoglobin liberated). It also occurs in rare cases that the antigen when injected into an animal whose tissue cells show no affinity for it or no proper receptors, will remain in the circulation for days and weeks without combining and producing any effect. The antigen, for example, a toxin, can be isolated from the blood in such a case in the same concentration and form as when it was injected. Some antigens have special affinities for certain tissues, as for example, tetanus toxin and nerve cells. In this case, however, the larger part of the antitoxin is produced by cells other than those of the nervous system. The production of antibodies for antigens probably occurs in the following way; the antigenous substances combine with the cells utilizing all the available receptors, leaving none open for food and thus perverting the general metabolism of the cell. In such cases there is a regeneration of these chemical receptors by the tissue cells which more than compensates for those with which the antigen has combined and as a result the cell discharges them (chemical substances) free into the body fluids.

The various antibodies are usually produced with more avidity by certain tissues than by others. Antibody formation may be of a strictly local character depending upon the point where the antigen is injected. For example, when abrin is placed in the eye, antiabrin is produced, but only in the eye so injected. In the majority of cases the antibodies are produced in some special tissue or tissues at a distance from the point of injection.

Following the injection of an antigen into the body of an animal there is always a decrease in the resistance of that body and a decrease in the antibodies produced followed in a short time by a marked increase in their formation. The former condition is spoken of as the "*negative phase*" and the latter as the "*positive phase.*"

Antibodies may be transferred from mother to young before birth, but only after fetal circulation is established. It has been positively demonstrated that antibodies are not transferred by the ovum or the spermatozoön directly. They are only carried from the blood of the mother and diffused through the placenta into the blood of the fetus. It has, however, been shown that the eggs of immunized chickens contain antibodies occasionally. This is "germ-cell transmission" and not true hereditary transmission. The transferred immunity or antibodies do not remain over two or three months in the bodies of the offspring after birth.

ANTITOXINS.—Antitoxins are so called because they combine with and render inert the soluble toxins. Antitoxins are produced for all the bacteria producing soluble toxins and for the toxic substances of a large number of other plant and animal cells. Antitoxins are the free chemical receptors of certain of the cells of the body. That is, they are chemical substances which have been thrown off from the cells of the body and in all probability were normally used for the purpose of taking up food substances although this is not positively known. These chemical substances are produced in excess of those actually needed by the cell due to a stimulation of the cells by the toxin. The antitoxins are labile substances which cannot be analyzed. They may be similar to euglobulins. They are composed of molecules of large size. Antitoxins when present in the body of an animal are protective and in many cases curative. According to Ehrlich, toxins are assumed to possess two chemical combining groups, one known as the *haptophore group* which combines with the cells and another known as the *toxophore* group which combines with the cell after the haptophore group has combined and this produces an intoxication of the cell. The haptophore group of the toxin molecule is thermostable (heat resistant) and the toxophore group is thermolabile (heat susceptible). When a toxin is injected into the body of an animal, or is produced during the process of an infection, the haptophore group combines with the cells with which it has an especial affinity and with the receptors (chemical substances which are unsaturated and open to combination with other chemical substances) of these cells. The chemical receptors of the cells with which the toxin-haptophore group combines are designated as *haptophile receptors*. It is probable also that the toxophore group of the toxin combines with other chemical receptors in the cell after the haptophore and haptophile groups have combined. These are designated as *toxophile receptors.* The haptophore receptors of the toxin having combined with the haptophile receptors of the cells, the toxophore group of the toxin then combines and intoxicates, stimulates or sometimes kills the cells depending on the affinity for the cells and the concentration of this group. In case the cell is not killed, it is stimulated and begins after a time to return to its normal functions. All the available receptors of the cells having been occupied and combined with, the cell sets about to generate new chemical receptors in order that food substances and other chemical substances may be taken up. The cells produce these haptophile receptors in excess, that is, there is over-compensation, and they are subsequently excreted into the lymph and blood. These haptophile receptors are in fact the chemical substances which we know as *antitoxins.* It is not only the cells with which both the haptophore and toxophore groups of the toxin combine because of special affinity, which make all the antitoxin, but cells which are widely separated from those which have an especial affinity for the toxin, also produce antitoxin. For example, tetanus toxin has an especial affinity for nerve tissue but this tissue produces little of the antitoxin. In this case most of the antitoxin seems to be produced in the spleen, lymph glands and bone marrow. The haptophore groups of the toxin have at least combined with these cells and stimulated them to the overproduction of haptophile receptors.

It has been mentioned that the antitoxins are *protective* to the body infected. The haptophile receptors (antitoxins) before they are thrown off combine with the toxin-haptophore and often the toxophore group does not have the opportunity for combining and killing the cells. This is in case there is no special affinity for the cells, as in the above-mentioned chief antitoxin-producing cells in tetanus. In such cases frequently all the available toxin is bound and very little is left to combine with the tissue with which it has an especial affinity, as is the case with tetanus toxin and nerve tissue. The antitoxins serve in this instance as protective substances. Furthermore, in case the antitoxin is excreted into the blood and lymph it serves in addition as a *curative* agent, all the toxin which is produced combining with all the available antitoxin in the circulation and none is left to combine with the cells of the body. The maximum affinity is always between toxin and antitoxin rather than between toxin and cell, if there is any antitoxin present. Antitoxins are prepared artificially and used for both prophylactic and curative purposes in the treatment and prevention of certain of the infectious diseases such as tetanus and diphtheria.

Antitoxins are also produced in the bodies of animals which are to all appearances immune to the toxins concerned. For example, the alligator is immune to tetanus but when tetanus toxin is injected into this animal tetanus antitoxin will be produced. In this case the haptophore group of the toxin has combined with certain of the cells of the body, but with such cells as give no opportunity for the toxophore group to combine, or have no affinity for this group. In the case of the alligator the nerve tissue seems to possess no chemical receptors for the toxin.

There are certain animals which are very susceptible to the action of certain toxins and which will not produce antitoxin when the toxin is injected. For example, the guinea-pig and the rabbit will not produce tetanus or diphtheria antitoxin when injected with small and gradually increasing doses of tetanus or diphtheria toxin. If the toxin is modified chemically by the addition of chemicals such as terchloride of iodine or by heat these animals may be immunized and will produce antitoxin. In this instance the virulence of the toxophore group is reduced and it is possible to inject the animals with more toxin, thus combining with more cells and finally liberating more antitoxin.

It should also be noted that animals of the same species vary in their power to produce antitoxin. The production of the product varies with the age and general condition of the animal and with the duration and the degree of toxicity of the toxin used. On account of this condition it is necessary to establish units or standards for determining the strength of antitoxins.

As stated in the discussion of natural immunity to toxins, there are some animals which when injected with toxins do not possess cells which have receptors open for chemical combination and as a result the toxin remains free in the circulation for varying periods of time. For example, as before stated, the frog is immune to tetanus and an injection of toxin will not produce any antitoxin. If tetanus toxin is injected into this animal it will remain in the circulation in the same form as injected and can be withdrawn after a few weeks or a month.

The Mechanism of the Neutralization of Toxin by Antitoxin.—At one time it was supposed that the antitoxin was but a toxin in a little different form but this has been absolutely disproven. The amount of antitoxin produced is much greater than the amount of toxin which is injected or produced during an infection.

The union between toxin and antitoxin is of a definite chemical nature. After these two substances unite the resulting compound is absolutely harmless and differs from both the toxin and the antitoxin in that it is much more stable.

In the beginning all experiments dealing with the union of toxin and antitoxin were performed in the body of an experimental animal (*in vivo*) but finally Ehrlich showed that they would act and combine equally well in the test-tube (*in vitro*) and could be studied much more easily.

The various toxins are neutralized by their antitoxins with varying rapidity. The concentration of these chemical substances, the temperature, the character of the medium in which they are placed, and the amount of electrolytic salts present, are accountable for the differences in length of time of combination. In the main these substances act like most chemicals and some of them show evidences of following the laws of multiple proportions. As a matter of fact the same laws which govern the union of toxin and antitoxin govern other antibodies and their antigens.

As before stated, toxins have a greater affinity for the free haptophile receptors of cells (free antitoxin) than for those still associated with the cells. Toxin and antitoxin will always combine, if the opportunity presents itself, before toxin and body cells will enter into chemical union. Furthermore, in certain instances, such as in diphtheria, when the toxin has been partially bound by the body cells and antitoxin is produced in sufficient quantities or is injected, the toxin-cell chemical union will be broken up and the toxin and antitoxin will combine. Obviously, antitoxins of this kind are very valuable in effecting a cure in certain infections. In the above-mentioned cases, the union between the toxin and the cell is comparatively unstable but this is not true in every case, as for example, in tetanus or lockjaw. In this case when once the toxin is combined with the cells of the nervous system and other body cells it is very difficult to break up the chemical combination by the addition of antitoxin. It requires exceedingly large doses and these rarely act efficiently. The union between toxin and body cells in this instance is very stable. We have here an explanation why tetanus antitoxin is of so little use for therapeutic purposes. It is, however, of use as a prophylactic when free toxin is being produced in the body. Diphtheria antitoxin is efficient both as a curative and prophylactic agent for the reasons which have been discussed above.

Antitoxin like toxin is fairly unstable and such agents as heat, light, and chemicals, affect it and reduce the potency. It may, however, be dried and kept for long periods of time in the dark. It is necessary in the commercial preparation of antitoxin and in its experimental study to have a unit or standard of measurement.

Units of Antitoxin.—In order to arrive at a standard it is necessary to accurately test a given antitoxin to determine the number of so-called antitoxic units it contains.

In the accurate study of the neutralization of the toxin by the antitoxin it is noted that adding fractional amounts of the antitoxin to the L° dose of the toxin* and injecting the resulting mixture into test animals (guinea-pigs), there is not a corresponding decrease in the toxicity as would be expected. The toxin is made up of various parts. The part just mentioned has a great affinity for the antitoxin but is not really toxic. Such parts of the toxin molecule are called *protoxoids*. The protoxoids compose about one-fourth of the amount of toxin necessary to saturate one immunity unit. After the one-fourth antitoxin is added to the L° dose of toxin the mixtures of toxin-antitoxin become less toxic for the experimental animals down to the point where approximately three-fourths of the amount of toxin necessary to saturate one unit of antitoxin has been used (three-fourths of L° dose). This fraction is *true toxin*. The toxicity of the mixture does not decline from this point when antitoxin is added up to one immunity unit, and it has been demonstrated by Ehrlich and others that this is due to another part of the toxin molecule which has a lesser affinity for the antitoxin than the true toxin itself and the protoxoid. This part of the molecule is called an *epitoxoid*, *true toxoid*, or *toxon*. The toxin molecule necessary to saturate one unit of antitoxin is, therefore, made up of *one-fourth protoxoid*, *one-half true toxin*, *and one-fourth epitoxoid*, *true toxoid* or *toxon*. The toxon is in certain instances slightly toxic and is supposed by some to be a secondary toxin and in certain diseases such as diphtheria, this substance has a weak affinity for antitoxin and is a possible cause of diphtheritic paralysis.

Antitoxins may be prepared for all the bacteria producing soluble toxins, such as *Bact. diphtheriæ*, *B. tetani*, *B. botulinus* and *Ps. pyocyanea*. Antitoxic substances may also be made for some of the products of other bacteria such as the *Strept. pyogenes* but these differ from true antitoxins. Antitoxins may also be prepared for the toxins of certain plant cells, such as abrin, recin, crotin, and for the toxins of animals, such as snake venom and spider poison. These substances are in the main similar to those produced by bacteria, although in certain characteristics they differ materially.

LYSINS AND BACTERICIDAL SUBSTANCES.—Under the lysins will be discussed those substances occurring in normal and immune sera which have the power of destroying and disintegrating bacteria, those disintegrating and liberating the hemoglobin of erythrocytes (red blood corpuscles) and those substances which have a lytic action on various body cells. The substances which act on the bacteria are called *bacteriolysins*, those acting on erythrocytes are called *hemolysins*, and those acting on the other body cells are called *cytolysins*. The mechanism of these lytic processes is quite complex. It should also be noted in this connection that there are certain substances which kill or seriously injure bacteria and body cells and do not actually disintegrate them. Such chemical bodies are designated respectively as *bactericidal substances* and *cytotoxins*.

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* The amount which just neutralizes one unit of standard antitoxin (United States Public Health Service).

The first observations in regard to bactericidal and bacteriolytic substances were made by Nuttall and later by Büchner. Büchner noted these substances in normal sera and other body fluids and named them *alexins* (Gr. to guard). He assumed that they were concerned in the immunity of the body. This is not necessarily true as certain blood sera are frequently highly bactericidal when the individual is relatively susceptible. This is true of human blood serum and *B. typhosus*. Furthermore, in certain instances the animal is immune to the disease and the serum is not in any sense bactericidal. This is the case with the dog and *Bact. anthracis*.

Pfeiffer a number of years ago observed that when *Msp. comma* of Asiatic cholera was introduced into the peritoneal cavity of the normal guinea-pig the bacteria underwent lysis. He also noted that the process was much more rapid in the immune guinea-pig. Pfeiffer had the idea in the beginning that lysis did not take place anywhere but in the body of the animal but later it was demonstrated by a number of men, among them Metchnikoff, that the lytic action would also take place in the test-tube (*in vitro*).

Bordét and others later showed that some normal sera possess the power of liberating the hemoglobin in red blood corpuscles. It was also shown that these hemolytic substances could be developed in the body of an animal if that animal were injected or immunized with a suspension of erythrocytes. The phenomenon of hemolysis is easily observed and studied and the amount of the hemolytic agent can be accurately determined as the amount of hemoglobin liberated varies accordingly. The mechanism of hemolysis and bacteriolysis correspond exactly and accordingly much about the latter process was first worked out by experimentation with hemolysins.

Lytic substances can be prepared for a large number of bacteria and for many body cells, as before stated. These substances may be markedly increased by the usual processes of immunization. Those substances which have the power to produce lysins are called *lysinogens* and are distinct antigens. The lysins are antibodies. The lysins may be prepared by injecting the experimental animal with the live cells, the dead cells, the disintegration products of cells and in some cases with the metabolic products of cells.

The Structure of Lysins.—Lysins and bactericidal substances have been shown to be composed of two distinct parts: one a thermolabile part known as the *complement* which is destroyed at a temperature of 55° to 60° for thirty minutes; and another part which is thermostable, known, on account of its double combining ability, as an *amboceptor.* This amboceptor will withstand heating to 60° for twenty-four hours but if the temperature is raised to 70° it is readily destroyed. If kept at ordinary room temperature or in the ice box amboceptors will remain active for years. According to Ehrlich, amboceptors are the free chemical receptors of the body cells. They are produced in the same way as antitoxins but differ from these bodies in that they have two combining groups, one known as the *cytophile* group with which the amboceptor combines with the bacteria or other cells, and the other known as the *complementophile* group, with which it combines with the complement. The complement seems to be a normal constituent of the blood serum and other body fluids. It is undoubtedly produced by the various body cells (leucocytes et al.) and during the immunization of animals with certain antigens it is probably increased only slightly, if any, in amount. The complement is supposed to be composed of two groups also, one a haptophore with which it combines with the amboceptor, and another a zymophore which readily produces the lytic action after the haptophore has combined with the amboceptor. On heating the complement the zymophore group is destroyed and a *complementoid* is produced. This substance is similar to a toxoid and will combine with amboceptor but no lysis will result. It is, however, the amboceptor, or so-called immune body, that undergoes the decided increase during the processes of immunization. It can be accurately demonstrated that the amboceptor must combine with the cell in question before the complement can combine. Cells, such as bacteria or erythrocytes, may be saturated with amboceptor and washed and when the complement is added and combined, lysis takes place. The complement will not combine with the cells under any circumstances unless amboceptor is present and has first combined with the cells. It is probable in a given serum or body fluid that there are several complements which may activate a variety of amboceptors. However, it has been shown that the same complement will activate a variety of amboceptors of certain kinds.

While the majority of lytic sera are thermolabile some have been noted which are thermostable to a certain degree. Hamilton has described such a serum resulting after immunizing animals to *Bact. pseudodiphtheriæ* and Horton has noted thermostable substances in normal rat serum which are lytic for *Bact. anthracis*.

Various sera have been noted which possess amboceptors for certain cells but are not lytic because they do not possess the necessary complement. For example, the serum of the dog contains amboceptors for *Bact. anthracis* but no complement. If in this instance a foreign complement such as that in guinea-pig or rabbit serum is added there will be lysis of the bacterial cells.

Occasionally the absence of complement is of benefit to the animal in question and may account for the seeming natural immunity. For example, the venoms of the poisonous snakes are nothing more than amboceptors and when these substances are injected into an animal body such as a hog, which does not possess the required complement, no lysis of the body cells takes place. On the other hand, should the animal, such as a rabbit or man, possess the necessary complement, as they do, lysis will take place.

Substances are sometimes present normally in sera which have the power of combining with the amboceptors which may be present, and prevent the latter from combining with the cells so that when the complement is added there will be no lysis. Such substances must be designated as *antiamboceptors*. These antiamboceptors (*antiantibodies*) may be developed in an animal by immunization with amboceptors of definite kinds. There are other substances which may also engage the amboceptors which cannot be called amboceptors in the true sense but they accomplish the same purpose and are, therefore, classed with these bodies.

The Deviation of the Complement.—The complement may be deviated in several ways and as a result lysis of the cells in question may be prevented.

Occasionally there is noted in sera normally substances which may combine with the complement and prevent this body from combining with the amboceptor. Such substances are called *anticomplements* and may be produced artificially by the immunization of animals with complement. Occasionally complement is absorbed by tissue cells and prevented from combining with amboceptor. In case there is an excess of amboceptors in a serum and only a small amount of complement, it may be deviated. In this case the cells will have taken up all the possible amboceptor and there will be an abundance of amboceptor free in the serum. It has been demonstrated that complement will combine with free amboceptor before it will combine with the amboceptor which has been bound to the cells. In this case all the available complement will be taken up by the amboceptor which is free and consequently there will be no lysis. This fact is of importance in certain infections where the development of bacteriolytic substances are of importance and necessary in effecting a recovery. The infectious microörganisms may not be destroyed for the above reason.

The Deflection of the Complement as a Test for Antibodies.—A very ingenious procedure has been devised for the testing of sera for unknown antibodies similar to bactericidal substances and lysins. The method of demonstrating the fixation of the complement was first worked out by Bordét and Gengou. The reaction is made use of in the test for syphilis which is briefly stated as follows: when the syphilitic antigen is combined with the supposed amboceptor in the blood serum of the suspected case of syphilis and a foreign complement, which has been accurately standardized, is added, this complement is bound and is, therefore, prevented from combining with red blood corpuscles, and a hemolytic amboceptor which may be added later. Hemolysis is, therefore, prevented. The general technic of the test is as follows: the syphilitic antigen is prepared by making an aqueous or alcoholic extract of the liver of syphilitic fetus or in several other ways. This antigen is supposed to contain protein and other chemical substances produced by the *Treponema pallidum*, the etiological microörganism of syphilis or similar substances to those produced by this microorganism. The blood serum of the suspected case of syphilis is heated to 56° for thirty minutes in order that the normal or immune serum complement may be destroyed. The new complement is supplied from normal guinea-pig serum. Before beginning the test it is necessary to have a rabbit immunized with some hemolytic antigen, such as sheep or human erythrocytes. There is developed in the serum of the rabbit the hemolysin for sheep or human corpuscles which when combined with these corpuscles will cause a liberation of hemoglobin. In the rabbit serum there are both hemolytic amboceptors and complement. It is necessary to heat this hemolytic rabbit serum to 56° for thirty minutes in order to destroy its complement and also it is necessary to find out accurately the amount of guinea-pig serum which will complement the resulting hemolytic amboceptor. This definite amount of complement having been determined, it is mixed with syphilitic antigen plus the syphilitic amboceptor, mentioned above, and allowed to incubate for one hour and thirty minutes at 37°. If the serum is from a case of syphilis the antibodies (amboceptors) will be present and combine with the antigen, and also the guinea-pig serum complement. The next step in the technic is to add to the above-mentioned mixture the hemolytic amboceptor and its antigen, sheep corpuscles. If the complement has been bound there will be none left to combine with the hemolytic amboceptor and no hemolysis of the sheep or human corpuscles will result. If the patient's serum does not contain syphilitic amboceptors or antibodies, the complement will not be bound and hemolysis will result. This test has been designated as the *Wassermann test* on account of the man first working it out in the case of syphilis, and has shown itself to be very efficient in the diagnosis of this disease in suspected cases. Many modifications of this test have been devised, some of which are very accurate.

The fixation of the complement may be made use of in the detection of any bacterial antibody, the procedure being approximately the same as above indicated and the hemolytic system used as an indicator as in the case of syphilis. The antigen, however, is different. When working with specific bacteria a suspension of bacterial cells in 0.85 per cent sodium chloride solution constitutes the antigen.

Cytotoxins and Cytolysins.—The names *cytotoxin* and *cytolysin* are used synonymously and are applied to those substances in sera and other body fluids which have the power of destroying cells other than erythrocytes. In a broad sense any substance destroying a cell would be cytotoxic but the terms are usually applied in the more limited manner, as above indicated.

Cytotoxins are produced in the same manner as other antibodies.

The immunization of an animal, for example, with renal (kidney) cells, produces in the blood serum of that animal a cytotoxin for the parenchymatous cells of the kidney. Cytotoxins can be produced for practically all the parenchymatous cells of the body. These immune bodies are not very specific and even careful experimentation leads to confusing results. For example, when an animal is immunized to kidney cells there is produced in the body of the immune animal cytotoxins for kidney cells and also cytotoxins in smaller amounts for other parenchymatous cells such as those of the liver. In the beginning it was supposed that the cytotoxins would be of value in the study of the physiological functions of organs and tissues. For example, a cytotoxin having been produced for the thyroid gland or adrenal gland it would be possible to inject this into another animal, destroy the gland, and then note the effect on the body. It was thought it might be possible to produce anticytotoxins which would be able to counteract the action of those cytotoxic substances which are produced in the body during the course of infections. However, the lack of specificity of the cytotoxin renders these procedures only theoretically possible. The fact that cytotoxins are produced for cells other than those used in the process of immunization indicates that there are similar chemical substances in the various cells.

There are *autocytotoxins* produced in the body. These probably result from the absorption of the products of disintegrated tissue cells. If no *anticytotoxins* for these autocytotoxins are produced, or they are not destroyed in some way, a very "vicious cycle" would result in that more of the specific cells of the organ or tissue used would be destroyed. Cytotoxins have been prepared for leucocytes and these substances are sometimes developed during the progress of an infection. The *leucocytotoxins* have perhaps been studied more than any one of others.

When ova are used for the purpose of producing cytotoxins, besides producing these substances in the serum of the immune animal, cytotoxins for spermatozoa of the same species are also produced, showing that these cells have some chemical substances in common.

Metchnikoff, following his idea that old age is due to a destruction of tissue by the mononuclear leucocytes, hoped that it would be possible to produce a cytotoxin for these cells. It is claimed by some that there are specific substances produced by the exhaustion of certain cells, that is, a toxin of fatigue. Weichardt has produced an antibody for this toxic substance which must be in reality an anticytotoxin.

It has been suggested that the cardiac hypertrophy in nephritis is due to the effect of a nephrocytotoxin on the peripheral blood-vessels causing increased diastolic pressure on the heart.

Another interesting substance has been produced and this is called syncytiolysin. It is prepared by immunizing animals with placental cells. It is claimed that this cytotoxin produces on injection symptoms similar to those noted in eclampsia and it has been suggested that the production of such a body in the pregnant woman from the placental cells may be the cause of this serious condition. Liepmann claims to have demonstrated placental constituents in the blood of pregnant women by means of the precipitin test. These bodies must be the antigen of cytotoxins. He states that when the blood of the pregnant woman is mixed with the specific syncytiolysin produced by immunizing an animal with human placenta a precipitate occurs. He suggests the possibility of a serum test for pregnancy. Abderhalden has reported some interesting results with the serum test for pregnancy and cancer. His findings cannot, however, be regarded as conclusive.

Cytotoxins are similar to bacteriolysins and hemolysins. They consist of amboceptors which are activated by the complement which is normally present in the serum or other body fluids.

THE OPSONINS AND PHAGOCYTOSIS.—It was shown a number of years ago that certain types of leucocytes and other body cells were capable of ingesting bacteria and other plant and animal cells. The mechanism of this process was not known until Wright and Douglas demonstrated certain substances in the blood serum and other body fluids which have the power of rendering the bacteria susceptible to phagocytosis. These substances are known as *opsonins* (Greek:—I prepare food for). The phenomena of the phagocytosis depend almost wholly on these special opsonins. Leucocytes which have been washed free from all serum will not take up bacteria except a few in rare instances. Bacteria which have been placed in contact with blood serum or other body fluids may be thoroughly washed, and then when they are placed in contact with the leucocytes, they will be taken up. The opsonin reacts chemically with certain substances within the bacteria, and so to speak, sensitizes them. Opsonins are present in many normal sera for the various bacteria. They may be produced in animals not containing them by the process of immunization with various antigenous microörganisms. Opsonins are destroyed at about 60° for thirty minutes, but there is some variation among them. When kept at 0° opsonins will remain active for several days, but at a temperature of the body, 37°, after the serum has been withdrawn, they rapidly deteriorate. Many opsonins have the structure of agglutinins and precipitins, although they bear some points of resemblance to antitoxins and complements. They possess two so-called chemical groups, a "combining group" by which they enter into chemical union with the bacteria and a "functional group" which really sensitizes the microörganism and makes it phagocytable.

It has been shown that the opsonins may be increased in the serum of the animal or injected individual by the injection of heated (60°) cultures of the specific etiological microörganisms. Such substances are called *opsonogens* or *vaccines* (bacterins). Vaccines are used to a certain extent in the treatment of the various pus infections due to the staphylococci and also in tuberculosis and pneumonia. It is supposed that the opsonins are produced in the subcutaneous tissues and in the muscles.

The Opsonic Index.—The concentration of the opsonins may be recorded in an individual in the following ways. Suppose the leucocytes of the infected individual take up a certain number of bacteria, say an average of 5, after counting 50 to 100 polymorphonuclear leucocytes. In this case the *phagocytic index* is said to be 5. Again, suppose the leucocytes of the normal individual take up 15 of the bacteria in question, the average after counting 50 to 100 leucocytes being always taken. The phagocytic index in this case would be 15. In order to determine the *opsonic index* of an infected individual the phagocytic index of the normal individual is taken as a denominator of a fraction and the phagocytic index of the infected individual as the numerator of the fraction. In the above illustration this would be $5/15$, $1/3$ or reduced to decimals 0.33+. The opsonic index, it can be seen, is somewhat of an indication of the resistance of the particular individual to the infecting microörganism in question. By the use of vaccines the opsonic index may be raised to at least 1.0 or even more, showing that the leucocytes are actively phagocytic and the opsonins increased in concentration in the blood serum. In such a case recovery would be indicated. When vaccines are injected in the treatment of infections the opsonic index has been shown to vary from time to time. Within a few hours after the injection the opsonic index falls below what it was at the time of the injection. This lowering of the index is known as the "*negative phase.*" Following the fall in the index there is a continuous rise to a point equal to what it was in the beginning and above this point. This rise in the opsonic index is known as the "*positive phase.*" The individual receiving the vaccine usually shows an increase in the symptoms during the "*negative phase.*" Obviously, it is necessary not to give a subsequent injection of vaccine until the patient is at the height of the "*positive phase.*" This can be best determined by determining the opsonic index.

Occasionally counts are made of the number of leucocytes which are actually taking up bacteria, disregarding the number of bacteria within the cells. The determination is always made on the basis of 100 and the per cent of leucocytes which are phagocytic is taken as the so-called *percentage index.* The percentage index also gives an idea of the resistance of the individual. It has been shown that in the practical work of treating infections with vaccines it is not absolutely necessary to determine the opsonic index or percentage index. The positive and negative phase may be determined fairly well by general clinical observations on the infected individual. Virulent bacteria are not readily phagocytized. For example, virulent streptococci and pneumococci are not phagocytized as easily as non-virulent forms. It seems in this instance that there is some toxic or poisonous substance produced by the bacteria that is antagonistic to the opsonins or perhaps an antiopsonin is formed.

The presence of opsonins in the body fluids of an animal is not absolute proof that such animal is highly resistant to infections. The resistance really depends on the activity of the phagocytes and in certain cases where the opsonins are high in concentration the phagocytes are not active. In other cases the reverse is true and in these cases opsonins and phagocytosis are of the utmost importance in the immunity of individuals. For example, in anthrax the immunity of the dog is due to opsonins and phagocytosis, while in the rat, although opsonins are present, there is no phagocytosis and immunity is due to antibacterial substances in the blood serum. In certain infections, such as typhoid fever, influenza, and uncomplicated miliary tuberculosis, there is a deficiency in leucocytes (leucopenia) and consequently even if the opsonins were concentrated and the bacteria sensitized there would be very little increase in the immunity from these causes.

Hemoöpsonins.—It has been demonstrated that very frequently opsonins for red corpuscles are present in the sera and body fluids of animals. Such bodies sensitize the red blood corpuscles and render them susceptible to phagocytosis by the polymorphonuclear leucocytes and the epithelial and other body cells. They are designated as *hemoopsonins.* Occasionally *iso-* and *autohemoöpsonins* are present in normal sera. For example, in human blood serum, it is probable that the process of red blood corpuscle destruction which takes place in the spleen may be referred to the action of these types of opsonins and various phagocytic cells.

AGGLUTININS.—Agglutinins are substances, present in the blood sera and body fluids of normal and immune animals, which have the power of producing a clumping and sedimentation of the microörganisms causing the specific infection or used in artificial immunization. The relationship of the agglutinins to the phenomena of immunity and the other antibodies which are produced during the process of infection and experimental inoculation is not known. One of the first agglutinins to be observed was that occurring in the blood serum in cases of typhoid fever and the agglutination reaction is now made use of in the diagnosis of this disease (*Widal test*). Agglutinins are specific substances and at high dilutions only cause a clumping of the microörganisms which give rise to their formation (antigens).

Normal Agglutinins.—Agglutinating substances, as above stated, are frequently found in normal sera. In this case no direct connection between their formation and specific microörganisms can be established. Normal human serum frequently contains agglutinins for *B. typhosus*, *B. coli*, *Bact. dysenteriæ*, and occasionally *M. pyogenes var. aureus* and *Msp. comma* in certain rare cases. Agglutinins for *B. typhosus* which are present normally in the serum may give rise to confusion when this test is used for the diagnosis of typhoid fever. It is, therefore, necessary to dilute the serum of a suspected case of typhoid fever at least one to forty or one to fifty times in order to exclude the normal agglutinins and the so-called coagglutinins.

The Production of Agglutinins.—Agglutinins may be produced artificially by the injection of bacteria, dead or alive, into the veins, subcutaneous tissues or peritoneal cavity. In rare cases they may be produced by feeding the bacteria, injecting them into the air passages of the lungs or by rubbing them into the skin. It is probable that the highest concentration of agglutinins results from the injection of dead bacteria. It is, however, necessary that these bacteria be not subjected to a temperature above 62°. Many pathogenic and non-pathogenic bacteria form agglutinins when injected into the body. The concentration of the agglutinins produced varies greatly. Very high agglutinating sera are noted, such as, for example, one in one million when *B. typhosus* is used and one in two million when *Msp. comma* of *Asiatic cholera* is used. Often two strains of the same organism will vary greatly in their power to produce agglutinins. Again, the concentration of the agglutinins in an infected animal varies from day to day, and in order to make an accurate observation it is necessary to make repeated examinations on subsequent days. For example, in typhoid fever the agglutinins one day may be thirty times as strong as on a subsequent day.

The Distribution of Agglutinins in the Blood.—As before stated, these antibodies are found in practically all the body fluids. They reach their highest concentration in all probability in the blood serum. In certain cases they are in high concentration in the milk. Agglutinins are also present at times in the sputum, tears, and the humors of the eye.

Inherited Agglutinins.—Agglutinating substances may be transferred from the mother to the offspring *in utero*. It has been frequently demonstrated, for example, that the offspring of mothers who have recently recovered from typhoid fever or who are infected at the time of the birth, have agglutinins in the body fluids. The same is true of the offspring of glandered horses. Notwithstanding the fact that the milk is frequently rich in agglutinins, these substances are not transferred to the offspring to any great extent by this means.

The Substances Concerned in Agglutination.—There are two distinct substances concerned in this reaction, one substance which is present in the serum or body fluids of the infected or immune individual, and other substances which are present in the microörganisms which are agglutinated. The substance in the serum, as before stated, is known as the *agglutinin;* the substance (antigen) in the bacteria or other microorganisms is known as the *agglutinogen.* When agglutinins and agglutinogens are combined together a new substance is formed which is designated as an *agglutinate*. As to the location within the bacterial cell of this agglutinogen (agglutinum) there is some dispute. Various authorities have stated that it is present in the cell wall or on the cell wall. Others have held the view that it is located within the cell protoplasm and in certain instances in the flagella. Without doubt, in certain cases this substance is excreted from the cell into the surrounding medium, as is shown by the fact that when filtrates of bacterial cultures are injected they frequently give rise to the formation of agglutinins. This agglutinogenic substance is specific and varies with the species. There are, however, very closely related substances of this character among some groups of bacteria. When these agglutinogenic substances are injected into the animal they frequently give rise to agglutinins which when combined with other members of this group will produce agglutination in low dilutions. Such a reaction and property is known as "*group agglutination*," and the agglutinins produced in such a case are known as *coagglutinins*. For example, the serum of the patient suffering from typhoid fever or of a person or an animal immunized with *B. typhosus* will produce an agglutination first of *B. typhosus*, but in addition, an agglutination of *E. coli*, *B. paracoli*, *B. paratyphosus*, and *B. enteriditis*. The agglutination of these last-named organisms, of course, will not be active except in low dilutions, and in order to exclude them satisfactorily it is necessary to dilute the serum to a higher point. This phenomena of coagglutination is due to the fact that there are some chemical substances (agglutinogenic) within these bacteria which are common to all and which give rise to the formation of agglutinins, which are chemically similar to each other in certain respects.

Structure of Agglutinins and Agglutinogens.—According to Ehrlich's conception the agglutinins are composed of two chemical groups, a haptophile or combining group with which it combines with the haptophore group of the agglutinogen and a zymophorous or agglutinophorous group which actually produces the agglutination. The agglutinogen is also composed of a combining group known as the haptophore group with which it combines with haptophile of the agglutinin. It is probable that this same haptophore group will combine also with various tissue cells and give rise to formations of agglutinins which are really free haptophile receptors of the tissue cells which have been acted upon by the agglutinogenic substance contained in the bacteria.

Agglutinoids.—It is possible by means of heat and chemicals to destroy the zymophorous group of the agglutinin leaving only the haptophile group. Such a substance is known as an *agglutinoid*, being similar to a toxoid. A temperature of not to exceed 60° to 70° is necessary to produce this substance. Agglutinoids will combine with the agglutinogen of the bacteria but they will not produce a clumping or an *agglutinate.* Occasionally in some fresh sera substances are found which have a greater affinity for the agglutinogen of the bacteria than the agglutinins have. Such substances are designated as *proagglutinoids* and are in this respect similar to protoxoids.

The Stages of Agglutination.—There are two distinct stages of the agglutination reaction. Neither of these stages can take place unless some salts or electrolytes are present. Sodium chloride is the common salt present. The first phase of the agglutination reaction is a union between the agglutinin and the agglutinogen of the bacteria. The second phase is the actual clumping of the bacteria. It is supposed that in this last phase the zymogenic group of the agglutinin is acting. In the first phase the haptophile group of the agglutinin is combined with the haptophore group of the agglutinogen.

There are some bacteria that cannot be agglutinated, as for example, *Bact. pneumoniæ* of Friedlander, and in rare instances *B. typhosus* cannot be agglutinated. It is possible, for example, to grow *B. typhosus* at a temperature of 42° and cause it to lose its power of producing agglutinins. Bacteria may also be modified chemically so that they will lose the power to produce agglutinins.

Agglutinins bear no relationship to bactericidal substances, antitoxins, opsonins or any of the other antibodies. They are both of use in the determination of species of bacteria when a known agglutinating serum is used, and they are also of use in determining the cause of infections where a known culture or agglutinogenic substance is used. The agglutination reaction is used in the diagnosis of typhoid fever, paratyphoid fever, glanders and dysentery.

Hemoagglutinins.—Agglutinating substances are sometimes produced for red blood corpuscles when these cells are used in the immunization of an animal. Such agglutinins when combined with the corpuscles produce a clumping which is known as hemoagglutination. The mechanism of the reaction is the same as that of bacterial agglutinins. It is possible that hemoagglutination is one important factor in the production of agglutination thrombi in certain infectious diseases such as typhoid fever.

PRECIPITINS.—Another group of substances, which are antibodies, is produced through the processes of immunization which have not been definitely connected with the phenomena of immunity. These substances are known as the *precipitins*. Precipitins may be produced for the protein substances of most bacterial cells and a large variety of other plant and animal cells, such as blood serum, milk and grains. They were first demonstrated in 1897 by Kraus, who noted that the bouillon filtrates of cultures of *B. typhosus*, *Bact. pestis*, and *Msp. comma* would cause precipitates when mixed with the blood serum taken from cases of these diseases. The precipitin reaction is definite and specific. The protein substance used in immunization or concerned in the infection is the only one which is precipitated when the antiserum is added. To the protein substance which produces the precipitins the name *precipitinogen* is applied. To that substance in the blood serum and body fluids of the immunized or infected animal or person the name *precipitin* is applied. The combination between the precipitinogen and the precipitin forms a new chemical substance known as a *precipitate*. Precipitin may be formed in various parts of the body, for example, in the parenchymatous cells of the organs and by the leucocytes. *Bact. diphtheriæ* will not act as a precipitinogen and will not produce precipitins. This is practically the only bacterium which will not yield these antibodies.

Normal Precipitins.—Precipitins for alien blood serums have been found in the organs and blood of seemingly normal animals. Normal precipitins for bacterial proteins have not been demonstrated to a certainty.

Mechanism of the Formation of Precipitins.—The mechanism of the formation of precipitins is similar to that of other antibodies. When the precipitinogen is injected into the body of an animal, it combines with certain of the body cells, occupying chemical receptors which otherwise would be used for the taking up of food products. As a result the cells produce new receptors and the number of these more than compensate for the ones already utilized. The chemical receptors are finally thrown off into the body fluids and form the precipitins. It is supposed that the precipitinogen contains haptophore receptors which combine with the haptophile receptors of the cells. When these haptophile receptors are regenerated and produced in excess, as before stated, they are thrown off into the body fluids and are really what we know as precipitins. Precipitins are produced most commonly for widely different or heterologous substances or sera (heteroprecipitins).

Autoprecipitins and Isoprecipitins.—It has been demonstrated that animals will not produce precipitins for their own protein substances. For example, if an animal is bled and injected with its own blood serum an antibody will not be produced. Therefore, *autoprecipitins* do not occur. Again it has been shown that only in rare instances do animals produce precipitins for members of the same species. For example, if an animal, such as a goat, is bled and the blood serum injected into another goat, it is only in rare cases that the second goat will produce an antibody which is capable of producing precipitation of the proteins in the first goat's blood serum. Such precipitins are known as *isoprecipitins* and occur only in a very small per cent of cases and with no regularity.

The Phenomena of Specific Inhibition.—When precipitins are heated to low temperature (50° to 60°) or are subjected to the action of light or certain chemicals, their power to produce a precipitate when combined with a precipitinogen is destroyed. The precipitin which has been heated becomes a *precipitoid* similar to an agglutinoid or a toxoid. Their ability to combine with the precipitinogen still remains. It is possible, therefore, for precipitoids to combine with all the available precipitinogen so that when fresh precipitin is added no precipitate will occur. This is known as *specific inhibition* and sometimes leads to very confusing findings in the study of these immune bodies.

Antiprecipitins.—When an animal has produced a precipitin in its blood serum due to the injection of the antigenous substance which in this instance is known as the precipitinogen, this precipitin, which is a definite antibody, may be used for the immunization of another animal and an *antiprecipitin* produced; that is, a body which will combine with the precipitin in such a way as to prevent precipitation when this substance is combined with the precipitinogen. This is then, in fact, an *antiantibody* and is practically the only example we have in immune reactions of such a substance. The antiantibody is the limit for antibody formation.

The Precipitinogen.—As before stated, the precipitinogen is any protein substance which will cause the formation of precipitins. Certain of the precipitinogens are composed of two groups, one which is thermostable and another which is thermolabile. Therefore, when these precipitinogens are heated and this thermolabile substance destroyed there results a substance which is exactly similar to the precipitoïd produced by heating the precipitin. Such bodies are known as the *precipitoids of the precipitinogen* in distinction from the *precipitoids of the precipitin.* These precipitoids retain their power to combine with precipitin, but no precipitate results on such combination.

The Precipitate.—When precipitin and precipitinogen combine it requires some little time before precipitation occurs. This is dependent upon the temperature (37° best) and certain other factors. The presence of the trace of organic acids materially facilitates this reaction. Furthermore, the reaction will not take place without the presence of certain electrolytes or salts.

Coprecipitins.—The phenomena of "group precipitation" does not occur as often as does "group agglutination." The bacterial precipitins are very markedly specific but some of the blood precipitins are not so specific. For example, in a case where two rabbits have been immunized, one with the blood serum of man and the other with the blood serum of the monkey, it is found that the serum of the rabbit immunized to human blood serum will precipitate monkey blood serum to a less degree, of course, than human serum. This is due to the fact that there are certain chemical substances in common in the blood sera of the monkey and man. There are other rare instances of coprecipitins which will not be discussed.

The Forensic Use of Precipitins.—The precipitins are of use on account of their great specificity in the identification of various albuminous substances. They have been used, for example, in the identification of bloods. Before the knowledge of the precipitins was available, the only means of determining one blood from another was by means of the microscopic examination of the corpuscles. If the corpuscles were in a good condition, it was possible, for example, to differentiate between a mammalian and fowl blood, on account of the nucleation of the corpuscles of the latter. By the use of the spectroscope it was also possible to determine whether a particular stain was blood or not. When it came to determining the exact species from which the blood came it was impossible. By means of the precipitins this can be done. For example, a stain which is supposed to be blood is carefully dissolved out in 0.85 per cent sodium chloride solution and placed in a sterile test-tube. A series of animals, such as rabbits, have been immunized to the various known blood sera and after immunization their sera are drawn off. These sera contain the precipitins for the various sera and corpuscles used in immunization. These precipitins are combined separately in small test-tubes with the salt solution preparation of the blood in question. A precipitate occurs when the corresponding precipitating serum is added. It is necessary, of course, to place these preparations in the incubator at 37°. By this method all types of mammalian blood may be separated from each other with the possible exception, as before stated, of monkey and human blood. In this instance it is necessary to make careful comparisons in order to determine the concentration of the precipitins. The precipitins may also be used in the identification of various meats and other albuminous substances such as eggs.

In some ways the precipitins resemble colloids and it has been shown that organic colloidal substances such as ferric hydroxide, etc., when in aqueous solution, may be precipitated by the addition of certain electrolytic salts. The precipitation occurs in this instance in a very similar manner to that of the organic precipitins.

The Theories of Immunity

Various theories have been proposed which attempt to account for the resistance naturally present in animals, and the resistance which may be artificially produced. One of the first theories proposed was the so-called *noxious retention theory* which held the view that in natural immunity there were natural noxious substances present in the body which prevented the growth of the infectious microörganisms. In acquired immunity it was supposed that, as the result of an infection, specific noxious substances were produced and consequently new infecting microörganisms of the same species as those producing the original infection were unable to grow. This theory has long been discarded. Another theory, for a time prominent, was known as the *exhaustion theory*. It was conceived that natural immunity was due to the fact that the body tissues did not possess the necessary food products for the invading microörganisms and that in acquired immunity these necessary food products were exhausted completely so that when a second infection was attempted none could possibly occur. This theory has also been discarded.

One of the most prominent theories is the one which has been held more recently, with some modifications, namely, the *chemical side-chain theory* of Ehrlich. It is claimed that tissue cells are made up of definite chemical substances which possess chemical side-chains which are open for chemical combination with other substances. It is by means of these chemical side-chains that food products are absorbed and assimilated by the cells. Furthermore, it is by means of these chemical side-chains that toxins and various poisons are absorbed by the cells. It seems to have been clearly demonstrated that as a result of the absorption by certain cells of the body of toxic substances, particularly bacterial toxins, that the cells are stimulated and produce or open up a excenss of these chemical side-chains for combination with various substances. It is conceived that if enough toxin (not enough to kill the cells), is assimilated by the cells the chemical side-chains which are definite chemical substances will be split off from the original cell compound and escape into the circulation. It is these escaped chemical side-chains which constitute the antitoxin or bactericidal substances. In the case of antitoxins, they possess a maximum affinity for the toxin and will combine with the toxin much more readily than the toxin will combine with the remaining chemical side-chains of the original cell compound. In the case of bactericidal substances they will combine with the bacteria and destroy them and liberate in this way the endotoxins which may subsequently combine with antiendotoxin (?) or tissue cells. Inasmuch as no antiendotoxins are ever produced, the presence of bactericidal substances in a large percent of instances is a detrimental factor. The production of antiendotoxins by some method or other is extremely desirable. Since the majority of our diseases are due to bacteria producing endotoxins, such a product would be of immense value in combating these infections. The chemical theory of Ehrlich explains many features of the phenomena of immunity. This theory has been the basis of nearly all of the preceding discussions on the various antibodies.

Metchnikoff suggested what may be called a *phagocytic theory* ot immunity. According to his ideas and those belonging to his school, the phagocytes, and principally the mononuclear and polymorphonuclear leucocytes, are concerned in immunity. He explains natural immunity to toxins on the basis of an increased toxin-absorptive power on the part of these cells for toxins. He explains natural antibacterial immunity as an increased power of phagocytosis for the invading microörganism by the leucocytes. He conceived that in acquired immunity to toxins these cells develop as the result of an infection or artificial injection of microörganisms, an increased power of absorption of toxin and the power of producing antitoxin, and that acquired immunity to bacteria producing endotoxins is due to the increased power of the phagocytes to ingest and digest invading microorganisms.

We find the best explanation for the phenomena of immunity in both the theories of Ehrlich and Metchnikoff. Undoubtedly certain forms or types of immunity are due to definite chemical substances known as antitoxins or bactericidal substances, while other types are due to the activity of the phagocytes.

CHAPTER III*

MANUFACTURE OF VACCINES

INTRODUCTION

On July 1, 1902, by Act of Congress, the Secretary of the Treasury, through the Public Health Service, was placed in control of all manufacture and sale of viruses, sera, toxins, and analogous products for human use. In order to manufacture and place such products upon the interstate market, any individual or corporation must secure a license from the Secretary of the Treasury through the Surgeon General of the United States Public Health Service. All candidates, before securing federal approval for such licenses, must allow federal inspectors the privilege of examining their laboratories, including the details involved in the processes of manufacture. At frequent intervals the Hygienic Laboratory purchases samples of licensed products upon the open market for the purpose of subjecting these to careful examination. If the samples of any products are found to be misrepresented as to potency or kind and amount of preservative, or if contaminating organisms are present, the manufacturer is immediately notified to recall such products from the market.

July 1, 1913, by a similar Congressional Act, the Secretary of Agriculture, through the Bureau of Animal Industry, was authorized to regulate the preparation and sale of viruses, sera, toxins and analogous products intended for use in the treatment of domestic animals.

The federal control of the manufacture of vaccines, sera, toxins and other biologic products related to specific infectious diseases, has reduced to a minimum the danger formerly involved in the use of such materials.

For one who is not a student of microbiology and preventive medicine, or not familiar with the technic involved in preparing biologic materials such as sera, tuberculins and vaccines it is difficult to realize the various steps necessary in the production of a safe and active product. The manipulations attending the preparation of the materials require large equipment, expensive apparatus and the services of trained laboratory experts. Animals which are used in the work must be quarantined and carefully inspected before being placed under treatment. The sanitary conditions of the laboratories, operation rooms and stables must be of the best.

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*Prepared by W. E. King

Infection of the animal organism is due to absence of natural or acquired resistance. The natural resistant forces of the animal body may be such that insusceptibility to specific microbial invasion is present; such a condition is called natural immunity. Acquired immunity, on the other hand, refers to a condition in which the natural susceptibility of the animal body is replaced by a temporary or permanent resistance toward specific microbial invasions. Acquired immunity may be active or passive, and may be brought about by application of a vaccine or an antiserum. The application of smallpox vaccine causes a specific reaction in the body, stimulating the development of natural defences against smallpox virus, and is followed by a condition of active immunity which is relatively permanent in duration. The use of diphtheria antitoxin, which contains the antibodies capable of neutralizing the diphtheria toxin molecules, results in passive immunity and affords temporary protection.

ACTIVELY IMMUNIZING SUBSTANCES (VACCINES)

Vaccines* are essentially weakened or modified viruses. Such materials as blackleg and anthrax vaccines may be used with safety, as a rule, only on animals which are free from the specific disease in question, because, theoretically, if a specific vaccine were applied to a patient suffering from a given infectious disease, the introduction of the attenuated organisms, or virus, would tend to augment the virulence of the infection. The action of such vaccines is preventive or prophylactic, and not curative.

ATTENUATED VIRUSES.—There are several methods which may be employed in attenuating or modifying viruses. The processes involve the treatment of viruses in such ways that they may be injected into the normal animal body without danger of producing serious symptoms or lesions, while at the same time sufficient specific, infectious qualities must be present to produce mild reactions. The successful vaccine is attenuated or modified to a degree which insures both safety and activity. The following are the more important methods used to modify viruses:

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* The term "vaccine" is also loosely applied to bacterins, bacterial vaccines or suspensions containing killed microörganisms.

Attenuation by growth at a temperature above the optimum. This is illustrated by Pasteur's method of preparing anthrax vaccine.

Attenuation by passage of the virus through some species other than the animal for which the virus is specific. Smallpox vaccine may be regarded as a virus modified by passage through a heifer or other animal.

Attenuation of the virus by drying at constant temperature. The Pasteur method of prophylactic treatment for rabies is based upon this method.

Attenuation by chemicals. The growth of certain pathogenic bacteria in the presence of weak antiseptics reduces their disease-producing activities.

Other methods of modifying viruses for the purpose of active immunization:

The simultaneous method or hypodermic application of the virus together with protective serum, as in hog cholera vaccination.

The association or combination of the specific pathogenic bacteria with those of other species as illustrated by the apparent restraining action of yeasts upon pyogenic bacteria and the antagonism which *Ps. pyocyanea* exerts toward *Bact. anthracis.*

The filtration of liquid cultures of pathogenic organisms, such as *Bact. diphtheriæ* or *B. tetani,* and the consequent separation of the organisms from the toxin. The toxin is used to immunize animals in the production of antitoxin.

The destruction of young living cultures of specific bacteria by moist heat at a temperature slightly above their thermal death-point. Heated cultures of *B. typhosus* and *Bact. pestis* are used as prophylactics against typhoid fever and bubonic plague.

There are many vaccines in practical and experimental use at the present time. Among those which are of recognized value as shown by extensive practical use and reliable clinical statistics, the following are the most important: smallpox vaccine, blackleg vaccine, rabies vaccine, typhoid vaccine and perhaps Pasteur's anthrax vaccine.

The simultaneous method, or injection of hyperimmune serum, together with the specific virus is used in vaccinating against hog-cholera, cattle plague (Rinderpest), anthrax and foot-and-mouth disease. Asiatic cholera, bubonic plague, tuberculosis, acne, pertussis, pneumonia, canine distemper, furunculosis, septicæmia hemorrhagica, gonorrhœa and various inflammatory processes are treated, practically and experimentally, by various methods of vaccination, either as prophylactic or curative measures.

Smallpox Vaccine.—The first experiments relative to vaccination against smallpox date back to 1796. Prior to that time, the only specific preventive method used in warding off this disease depended upon the inoculation of healthy individuals with smallpox virus from a mild case of the disease. The present method of vaccination utilizes cowpox virus as the protective material. It has not been conclusively determined that cowpox in cattle and smallpox in man possess intimately related causative factors, but notwithstanding, abundant evidence proves the efficacy of cowpox virus as a specific prophylactic against smallpox in man.

In the practical preparation of smallpox vaccine, the virus or "seed" is first secured by removing the pulp from the vesicles which appear on infected heifers. Most laboratories which engage in this work use a stock mixture of cowpox virus which originated from spontaneous cases of cowpox, and which is known to produce active smallpox vaccine.

Great care is exercised in the selection and preparation of animals used in making the vaccine. Heifers (calves or yearlings) are most frequently used in this work, older cattle being employed in a few European laboratories. When first purchased these animals are placed in a detention stable where they are inspected by a qualified veterinarian and carefully tested for tuberculosis. If, after several weeks' quarantine, they are passed as healthy in every way, they are admitted to the vaccine laboratory after their bodies have been scrubbed with soap and water and a weak antiseptic solution.

The operating room and propagating ward should be constructed with a view to thorough cleanliness. Concrete floors, enameled walls and ceilings and simple sanitary apparatus should characterize the appointments. Floors, walls, ceilings and all equipment of these rooms should be carefully cleansed with disinfectant solutions at frequent intervals.

After the heifers are prepared for the work, they are inoculated with the seed virus. The animal under treatment is placed on a special operating table, the ventral surface of the body is shaved and cleansed, and, with a sterile instrument, is scarified in parallel straight lines over the greater portion of the abdomen and inner surface of the flanks. The stock virus or "seed" is inoculated in the scarified areas and the animal is released and placed in the propagating room. During the process of propagation of the vaccine all possible precautionary measures should be used to avoid the introduction of contaminating bacteria. It is important that an attendant be constantly present, day and night, whose duty it is to remove instantly all dirt and fæces and keep the room as clean and free from microbial contamination as possible.

At the expiration of from seven to nine days, characteristic vesicles will have developed on the inoculated areas. These are filled with a thick, sticky, purulent material. At this time the animal is removed to the operating table, the field of operation is washed with sterile water and the contents of the vesicles removed with a sterile curette. According to regulations of the Federal Government all animals used in this work must be slaughtered before the vaccine is removed and later submitted to careful autopsy. After removing the pulp, or vaccine, the material is handled under aseptic precautions and mixed with about 50 per cent glycerin, which serves as a preservative. Small portions of the material are then inoculated into guinea-pigs for safety tests and the product is placed in the refrigerator. Under the influence of the glycerin extraneous microbial contamination gradually disappears. Potency tests of the vaccine are conducted by the cutaneous application of the vaccine on calves, rabbits or on slightly scarified, scrotal surfaces of guinea-pigs. In addition to the safety and potency tests, inoculations are made into culture media which are placed under both aerobic and anaerobic conditions to insure the absence of harmful bacteria. For the detection of the presence of *B. tetani* the product is submitted to a special test by transferring 1 c.c. into a quantity of glucose beef bouillon or other special culture media, placing the culture under anaerobic conditions and incubating at body-temperature for about ten days. After the incubation any resulting growth is removed by filtration and the filtrate is injected into guinea-pigs.

The absence of symptoms in the treated animals shows that no tetanus toxin has been elaborated in the culture medium and therefore that the vaccine does not contain *B. tetani*.

After the tests are completed, the product is distributed under aseptic conditions, in small, sterile, capillary tubes sealed in sterile, glass containers, properly labeled, dated and kept in the refrigerator until placed upon the market.

If kept in a cold dark place, smallpox vaccine retains its protective activity for a considerable period. Under the influence of heat and light it rapidly deteriorates. For this reason it is difficult to ship the vaccine to tropical countries. Under suitable conditions the product should remain active for a period of about one year.

BLACKLEG VACCINE.—The production of blackleg vaccine depends upon the use of a virulent culture of *B. anthracis symptomatici* (*B. chauvœi*, *B. gangraenœ emphysematosœ*). A heifer is inoculated with a small portion of the virus and rapid, acute symptoms are usually produced. Death usually supervenes in about three days. The carcass and ward are thoroughly disinfected, the body of the animal is suspended, and, after again carefully disinfecting the outside of the body, portions of the skin are removed and the muscular tissue is inspected. Those areas of the muscles which show the dark color, gaseous formation and characteristic lesions of blackleg, are removed to the laboratory and examined microscopically for the presence of the specific organisms. After the muscle is freed from the gross connective tissue, it is suspended in strips or finely chopped, and allowed to dry spontaneously. It is then ground and sterile water is added until the mass becomes pasty or putty-like in consistency, after which the material is placed in small shallow pans and attenuated by drying at temperature of 85° to 100° for six or seven hours. In preparing the "single vaccine" most laboratories attenuate the virus by drying at an average temperature of about 90° for six hours. In addition to the aseptic precautions observed in conducting the above processes, microbial contamination is practically eliminated by the devitalization and probable death of any extraneous vegetative forms during the attenuation process.

Blackleg vaccine (single) is tested, according to the method recommended by the Bureau of Animal Industry, U. S. Dept. of Agriculture, as follows: A series of eight guinea pigs are injected intramuscularly with the vaccine under test; three each with three-fourths the dose for cattle, three each with one-half dose and the remaining two with one-third dose.

Temperatures of the test animals should be recorded for three subsequent days. Vaccine of proper strength is indicated when thermal reactions occur in practically all the test animals together with local reactions in some instances. None of the animals in the series should die.

As an additional test for potency a heifer may be injected subcutaneously with one dose and a few weeks after the vaccination the animal may be exposed to the disease by receiving an injection of the virulent living organisms. If the animal remains normal the activity of the product is indicated. In order to test the vaccine in regard to safety, heifers may be injected with several doses each. The absence of severe disturbances shows that the material may be used without danger.

For the purpose of eliminating possible danger from the use of blackleg vaccine a "double vaccine" may be employed. This consists of two vaccines, each possessing different degrees of attenuation, which are controlled by the degree of heat and the period of time used in attenuating the organisms in the affected muscle tissue. When the final product, either single or double blackleg vaccine, is ready for use it is usually distributed in the form of a powder, prepared threads or small pills. The latter, first suggested by Houghton in 1898, are injected hypodermically.

Blackleg Aggressin.—Blackleg Aggressin is a tissue extract containing the immunity-producing substances which are naturally present in the tissue of calves, dead from acute blackleg. The tissue juices of calves dead from the disease as a result of inoculation with pure strains of blackleg bacillus are recovered from the affected tissues and rendered free from the blackleg organism and extraneous contamination by filtration. The dose for cattle is 5 c.c.

Blackleg Aggressin may be tested for activity or potency by the injection of a series of guinea-pigs as follows: Two pigs each are injected with 1, 2, and 3 c.c. of the aggressin under test. After ten days the series of pigs together with two controls are injected with twice the minimum lethal dose of virulent blackleg culture or virus. The control pigs should die in the usual time and at least four of the series of six vaccinated pigs should live. Blackleg Aggressin may also be subjected to potency test by injection of calves with 5 c.c. each, or with varying doses. If prepared according to standard methods from carefully selected strains of blackleg bacillus, the calves receiving dosage of 3 to 5 c.c. should live, following the inoculation of the animals with virulent blackleg virus, ten days after receiving the aggressin.

Blackleg Filtrate.—Blackleg filtrate is a cultural product containing the metabolic substances resulting from the growth of the blackleg bacillus in liquid culture media. After incubation, usually about seven days, or until optimum growth has taken place and the cultures are checked for purity, a preservative is added and the organisms are removed by passage through Berkefeld filters. The method of testing for potency is similar to that for blackleg aggressin.

Rabies Vaccine.—The successful preventive treatment for rabies, or hydrophobia, resulted from the brilliant researches of Pasteur. The method devised by Pasteur in 1885, with some modifications, continues to be the only practical, specific preventive treatment for rabies. This treatment consists of a series of vaccinations, each vaccination involving the application of rabies virus having a known degree of attenuation. In each succeeding application of modified rabies virus the patient receives increasingly more virulent material until finally active immunity is acquired and subsequent attack from the disease is successfully resisted.

The preparation of rabies vaccine begins with the attenuation of a virus having a known degree of virulence. The material may be secured from an ordinary case of "street rabies." A dog suffering from the disease is killed and a small portion of the brain removed. The brain tissue is emulsified in sterile water or salt solution and a few drops of the material thus suspended in liquid, are injected subdurally into a rabbit. This may easily be accomplished by trephining the skull, after anæsthetizing the animal, and with a small syringe inoculating a few drops of the suspension just under the exposed dura mater. The inoculation of ordinary rabies virus usually produces symptoms of "dumb rabies" and the death of the animal in fourteen to eighteen days. In order to increase the virulent properties of the same strain of rabid material, it is transmitted from rabbit to rabbit by subdural inoculations until the incubation period is shortened to about six days. Experience has shown that when the virus has reached its maximum degree of virulence for the rabbit, the animal shows symptoms on the sixth or seventh day after inoculation. When the virus attains this degree of virulence it is called "fixed virus" and may be used in the preparation of the vaccine. The "fixed virus" or spinal cord of the rabbit which has succumbed to the disease in six or seven days, is removed aseptically and placed in a special drying chamber. The cords are suspended over caustic potash and dried at a temperature of 23° for a period of from one to ten or fifteen days.

The treatment of the patient consists in the hypodermic application of the "fixed virus" which has been attenuated by drying. The exact nature of the vaccine used in the initial vaccination and the time consumed in the series of injections depend, to some extent, upon the case in hand. Frequently, the patient is first vaccinated with a suspension of a spinal cord which has been attenuated by drying for fourteen or fifteen days. On the succeeding days of the treatment use is made of the suspension of spinal cords, which have been less and less attenuated. The treatment usually lasts about twenty days or until the patient has received an injection of the least attenuated "fixed virus."

It is very important, when one is bitten by a rabid animal, that the Pasteur treatment be begun as early as possible, in order that active immunity may be secured before the expiration of the incubation period. In many of the larger cities of the United States, for some time, laboratories have been maintained for the purpose of administering the Pasteur treatment. More recently, commercial laboratories have developed methods of preparation and distribution, so that any physician may purchase the vaccine and administer it to his patients.

Hogyes* substituted dilutions of the "fixed virus" for the dried spinal cords. For the initial treatment, a few cubic centimeters of a 1:10,000 dilution were used. In the succeeding injections graduated dilutions were employed. While the work of Hogyes has been confirmed by other investigators, the method is not generally regarded as possessing the safety of the original Pasteur treatment.

Harris † has devised a simple method of preparing the vaccine by freezing the infected spinal cord of the rabbit with CO_2 snow, and then drying the material *in vacuo* over sulphuric acid at a temperature of 10° to 15°.

* Hogyes, Acad. des Sciences de Budapest, Oct. 17, 1897.

† Harris, Jour. Infect. Dis., 1912, 10, p. 369.

The product is kept in the refrigerator in hermetically sealed vials. It is claimed that the material so prepared maintains its original strength or infectivity several months.

Cummings'* method consists in the dialysis of the rabic material in standard suspensions. Dialysis for twelve to twenty-four hours possesses the advantage of destroying the infectivity of the virus, without disturbing its immunizing properties.

DORSET-NILES (HOG-CHOLERA) SERUM.†—To prepare the material for this process of immunization it is first necessary to secure a virulent strain of hog cholera virus. This may be obtained from any typical outbreak of the disease. A specimen of blood may be drawn, aseptically, from the carotid artery of a pig suffering from the disease, and tested for activity. Frequently a given strain of virus may not produce the acute form of hog cholera. In attempting to raise the virulence of a relatively weak virus it may be passed through a series of young pigs until it uniformly produces symptoms after four to six days' incubation and death in less than fifteen days. None except a virus having this degree of activity should be used in manufacturing the hyperimmune serum.

The virulent blood used in the process of hyperimmunization should be obtained from susceptible pigs weighing from 25 to 50 kg. (50 to 100 pounds) each. The animals to be used as the "hyperimmunes" should be healthy hogs, each weighing from 100 kg. to 150 kg. (220 to 330 pounds) and possessing either natural or acquired immunity to the disease. The blood is best secured from a diseased pig by suspending the animal with the head down covered with a shroud wet with a disinfectant solution. The neck is shaved and disinfected. A small incision is made on the median line through which a specially devised bleeding knife, properly sterilized, is introduced. The blade of this knife severs the large vessels at the base of the heart and the blood flows through the hollow handle into sterile containers. After the blood is obtained it is defibrinated, the serum separated from the clot, and the clot discarded. The number of pigs necessary to furnish sufficient virus for the hyperimmunization of one hog depends upon the weight of both the virus pigs and the immune hog.

The immune hogs may be hyperimmunized either by the "slow" or "quick" method. In the former, now seldom used, the animals receive several injections at intervals of every few days, each succeeding dose being increased in proportion to the weight of the animal. In the "quick" method the virus is injected in one large dose, the amount being determined by the weight of the animal. The virus may be injected intramuscularly, intraperitoneally or intravenously, the latter method now being used almost exclusively. Ten days to two weeks after the hyperimmune hog has received the last injection of virus, the animal is ready for bleeding. When bled from the tail, the end of the appendage is severed with a sharp instrument, several hundred cubic centimeters of blood are collected aseptically, defibrinated, a preservative added and the material placed in the refrigerator. This process is repeated several times, at intervals of one week to ten days, when the animal is ready for final bleeding.

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* Proceedings 15th International Congress on Hygiene and Demography, Washington D. C., 1912.

† U. S. Bureau of Animal Industry, Bull. No. 102.

By this procedure all the blood is secured from the animal according to the method described for bleeding virus pigs. The "slaughter" method, used in many laboratories, consists of only the final bleeding, thus eliminating tail bleedings. As a rule the different lots of serum representing the different bleedings from several hyperimmune hogs are mixed and the whole subjected to test. In order to test the potency of the product eight susceptible pigs, each weighing about 23 kg. (50 pounds) are inoculated subcutaneously, each with 2 c.c. of virus. Six of these pigs are simultaneously injected with graduated doses (15 to 25 c.c.) of the serum under test. If the hyperimmune serum possesses potency the test pigs should remain in a normal condition throughout the test, except for the presence of thermal reactions and slight clinical symptoms, while the two control pigs should show severe symptoms in five or six days and should die in less than fifteen days.

The practical method of treatment in the field consists in the simultaneous injection of the hyperimmune serum and virus (double treatment), into healthy hogs for the purpose of immunization. The amount of hyperimmune serum which should be injected varies from 30 c.c. to 90 c.c., depending upon the weight of the hog to be treated. Thus, a hog weighing 34 kg. to 45 kg. (75 to 100 pounds) usually receives 40 c.c. of serum, together with 1 c.c. of virus. The usual dose of virus for hogs above 34 kg. (75 pounds) weight is 2 c.c. For pigs weighing less than 23 kg. (50 pounds) ½ c.c. of virus should be injected.

Anthrax Vaccine.—While several methods have been used in vaccinating against anthrax, probably the most important, at present, is that devised by Pasteur. This method consists in the use of cultures which have been attenuated by growth on artificial culture media at temperatures above the optimum. The inoculation of such attenuated cultures into healthy animals results in active immunization.

The stock culture of *Bact. anthracis* is usually obtained from the blood of a typical case of anthrax. The culture is transferred to agar or broth and incubated. Two vaccines are prepared, the first being less active than the second. Vaccine No. 1, is made by placing in suspension in sterile, physiological salt solution or other liquid, the anthrax organisms which have been grown at a temperature of 42° for a period of fifteen to twenty days. Vaccine No. 2 consists of a similarly treated culture of *Bact. anthracis* which has grown at a temperature of 42° for ten to fifteen days. Tests of both vaccines for activity and safety are made by animal inoculations. Vaccine No. 1 should kill white mice but should not cause fatal results in guinea-pigs or rabbits. Vaccine No. 2 should prove fatal for both white mice and guinea-pigs, but not for rabbits.

Healthy animals are first injected subcutaneously with about 1 c.c. of vaccine No. 1. Several days or a few weeks after the application of vaccine No. 1, the second vaccine is injected. A severe reaction and sometimes death follows the use of the vaccine. Accidents of this kind have resulted from careless methods employed in standardizing and administering the vaccine. The most important objection to Pasteur's anthrax vaccine is due to the danger involved in the use of the living, attenuated anthrax organisms.

Scalvo* advocates the use of the serum from animals actively immunized to anthrax. This method may be employed either in the form of the immune serum alone, or the immune serum and anthrax culture simultaneously.

Eichhorn † advises the use of antianthrax serum for curative purposes, and the simultaneous treatment with antiserum and a carefully standardized spore vaccine as a preventive. When vaccine alone is to be employed Eichhorn prefers the spore vaccine rather than the ordinary Pasteur vaccine.

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* Scalvo, Centralbl. f. Bakt., 1899, 26, p. 425.

† Eichhorn, Bull. No. 340, U. S. Dept. of Agr.

Tuberculosis Vaccine.—Among the experimental products for the prevention of animal tuberculosis may be mentioned von Behring's "bovo-vaccine." The technique involved in the preparation of this vaccine is not generally known. Romer* describes the material as being composed of the living tubercle organisms which are dried for a period of thirty days in sealed glass tubes. After this process of attenuation the organisms are injected, in carefully graduated doses, into healthy calves. Field tests which have been made upon calves with bovo-vaccine indicated unsatisfactory results.

In human practice various tuberculins prepared both from the bouillon culture and from the cellular elements of *Bact. tuberculosis* are used as therapeutic and diagnostic agents. Products containing the cellular elements are similar in nature to bacterial vaccines.

Bacterial Vaccines (Bacterins)

Opsonins may be defined as the elements in the blood or body fluids which are capable of modifying invading bacteria in such a way that they become ready prey to the leucocytes. In the presence of opsonins, therefore, phagocytic activity is increased. Opsonins are apparently distinct from agglutinins, lysins, and other analogous substances, because different degrees of heat are necessary for their destruction. Moreover, a given serum may agglutinate, or may exert lytic action, without possessing opsonic activity.

Wright and Douglas first advanced the theory of opsonic action, and suggested that the subcutaneous injection of a given species of bacteria, killed by heating, caused the blood of the treated individual to exert greater opsonic activity toward the species of organisms in question. The results of the work of others proved to be confirmatory.

To prepare a bacterial vaccine, the specific organism is isolated and after being grown for twenty-four hours or longer at a temperature of 37°, it is emulsified in sterile physiological salt solution, heated at approximately 60°, or killed by the use of chemical agents, standardized as to the number of bacteria present in 1 c.c. of the emulsion, and a preservative added.

If the patient and attending physician are conveniently situated in respect to a laboratory, the "opsonic index" may be taken before and during the treatment. This consists in the determination of the average number of the given species of bacteria ingested by the leucocytes of the patient, as compared to that which the leucocytes of normal blood are capable of destroying. It is usually found that immediately following the injection of specific bacterial vaccine there is a "negative phase" during which the leucocytes of the patient destroy a smaller number of bacteria. This is followed by a "positive phase," characterized by more active phagocytosis. For practical purposes the determination of the opsonic index is unnecessary as the clinical reaction following the injection of a given vaccine indicates correct dosage and progressive results of the treatment.

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* Romer, Beitrage z. Exp. Therapie, 1904, 7.

The use of bacterial vaccines has yielded excellent results especially in the curative treatment of furunculosis, acne, sycosis, puerperal infection, arthritis and other affections caused by pyogenic organisms, and in chronic infections of the genito-urinary tract. The material may be used in the form of "autogenous" or "stock" vaccines. An autogenous (personal) bacterial vaccine is one prepared from a culture of the specific organism isolated from the case under treatment. Bacterial vaccines, prepared from stock cultures of the specific organisms, may be manufactured and kept until needed for use. Some of the more common stock bacterial vaccines represent the following organisms alone or in various combinations: *Strept. pyogenes*, *M. pyogenes var.* (*albus*, *aureus* and *citreus*), *M. gonorrhœa*, *Bact. pertussis*, *M. pneumoniæ*, and *B. coli communis*.

The study of bacterial vaccines occupies a position of so much importance in preventive medicine and therapeutics that many new combinations of killed bacteria are being constantly added to the list of experimental products. Some of these have been under observation for a considerable time and are recognized as possessing valuable properties.

TYPHOID FEVER.—The typhoid bacterial vaccine of Wright* is generally accepted as a valuable preventive against infection. For prophylactic treatment, a series of three hypodermic injections (500,000,000, 1,000,000,000 and 1,000,000,000) of killed typhoid organisms are usually given.

Typhoid-paratyphoid vaccine (Bacterin) is frequently used, consisting of *Bacillus typhosus* 1000 million, *Bacillus paratyphosus A* 500 million, and *Bacillus paratyphosus B* 500 million.

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* Wright, Jour. of Hyg. 2, 1902, p. 385.

PNEUMONIA.—Experiences of recent years, especially in the army, have proved the value of pneumococcus vaccine, particularly as a prophylactic agent. The usual dose consists of 500 million killed pneumococci.

INFLUENZA-PNEUMONIA.—Among the various bacterins which were prepared and used experimentally during the influenza-pneumonia epidemic of 1918–1919, the preparation suggested by Rosenow[*] of the Mayo Foundation has found rather extensive use. This bacterin is composed of carefully selected strains of pneumococci, types 1, 2, 3 and 4, *Strept. hemolyticus* and *B. influenzæ.*

CANINE DISTEMPER.—Ferry,[†] corroborated by Torrey[‡] and McGowan[||] found this disease to be primarily an infection of the upper respiratory tract due to a small motile bacillus, *B. bronchisepticus.*

Ferry and Torrey proved that suspensions of killed cultures of this organism will immunize dogs against experimental inoculations as well as against the ordinary street infection. The bacterial vaccine is being used for prophylactic purposes in graduated doses of 200, 400 and 600 million bacteria per c.c., given at intervals of about five days.

ASIATIC CHOLERA.—Two methods of vaccination against this disease have been proposed and statistics which relate to field tests show positive results with both. The method of vaccination resulting from the work of Haffkine[§] depends upon the use of cultures of the spirillum of Asiatic cholera, attenuated by growth at temperatures above the optimum. Vaccines of different strengths are used. Kolle[¶] has proposed the use of heated (killed) cultures of the organism. Strong[**] has developed a vaccine for Asiatic cholera consisting of the filtrates from suspensions of killed and living *Msp. comma* (*Sp. choleræ Asiaticæ*). This vaccine is standardized in terms of immunity units, one unit "equaling the amount of immune serum which will protect a guinea-pig of 250 g. weight against the intraperitoneal inoculation of ten times the fatal dose of living cholera organisms."

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[*] Rosenow, E. C., Jour. A. M. A., Vol. 72, No. 22, p. 1604.
Rosenow, E. C. and Sturdivant, B. F., Jour. A. M. A., Vol. 73, No. 6, p. 396.

[†] Ferry, Am. Vet. Rev., 1910, Vol. 37, p. 499. Jour. of Infec. Dis., 1911, vol. 8, p. 399.

[‡] Torrey, Jour. of Med. Research, 1913, Vol. 27, 291.

[||] McGowan, Jour. of Path. and Bact., 1911, Vol. 15, p. 372.

[§] Haffkine, Brit. Med. Jour., 1895, 2. pp. 727, 1509.

[¶] Kolle, Deut. med. Woch., 1897, 23. p. 4.

[**] Strong, Philip. Am. Med., 1903, Vol. 6, p. 272.
Strong, Philip. Jour. Sci., 1907, Vol. 2. p. 155.

Bubonic Plague.—Practically the same methods of procedure have been followed in the experimental vaccination against bubonic plague as in the case of Asiatic cholera. Cultures of the plague bacillus, killed by heating at a temperature of 60° for one hour, have been used with success.

Sensitized Vaccine

Besredka* has developed modified bacterial vaccines known as sensitized vaccines. In the preparation of these the living microorganisms are brought into contact with the homologous antisera and the mixtures allowed to stand for approximately twenty-four hours at room temperature. The organisms are then removed by centrifugalization, washed and placed in suspension. The remaining processes of manufacture are similar to those employed in the preparation of ordinary bacterial vaccines.

Besredka and his associates explain the advantage of sensitized vaccines by the fact that in such preparations the microörganisms, by reason of having been in contact with homologous antisera, are sensitized with specific amboceptors. Therefore, the sensitized organisms are capable of immediately combining with complement, when introduced in the blood of the patient, and prompt immunization should follow.

Both living and killed sensitized microörganisms have been used experimentally, Besredka† having advocated the use of the former as devoid of harmful properties and more certain of successful results. Sensitized vaccines are still in the experimental stage, and their advantage over the ordinary bacterial vaccines is at present a debated question.

Toxin—Antitoxin Mixture.

Babes,‡ in 1895, first suggested the use of diphtheria toxin and antitoxin mixture as a method of immunization against diphtheria. Through the work of Park and Zingher|| and others who preceded, this method is being adopted in practice, especially as a means of prophylaxis against diphtheria in schools and hospitals. The mixture consists of active diphtheria toxin and antidiphtheritic serum in the proportion of 80 per cent. of the L + dose of toxin to one unit of antitoxin.

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* Besredka, Compt. Rend. de l'Acad. Sci., 1902, 134, p. 1330.

† Besredka, Bull. de l'Inst., Pasteur, 1910, 8, p. 241.

‡ Babes, Bul. Acad. de Med., Paris, 1895, 34, p. 216.

|| Park and Zingher, Jour. A.M.A. 1915, 65, p. 2214.

CHAPTER IV*

THE MANUFACTURE OF ANTISERA AND OTHER BIOLOGICAL PRODUCTS RELATED TO SPECIFIC INFECTIOUS DISEASES.

The principles involved in serum therapy are those of passive immunization. Therefore, the employment of an antiserum as a preventive or curative measure is an attempt to supply the patient with certain specific substances which are capable of neutralizing and destroying the specific toxic materials and pathogenic microörganisms. Presumably, the patient receives nothing in antisera which stimulate the development of protective bodies. Active immunity does not follow as in the case of vaccine treatment. As the result of serum treatment, the patient enjoys relatively temporary protection (preventive treatment), or cessation of pathologic processes (curative treatment), because of the application of specific antisubstances. The substances contained in the serum are developed in the blood of some other species, as the horse, through repeated injections of the animal with the specific organism in question or its toxin.

Antisera are divided into antitoxic and antimicrobial sera. An antitoxic serum is one possessing substances which, in contact with the specific toxin, unite with it, forming chemically stable and physiologically inert compounds. Under the term "antitoxic serum," in addition to antidiphtheritic and antitetanic sera, are grouped antisera for the soluble toxins of *B. botulinus* (specific meat poisoning), abrin, ricin and crotin (plant toxins), snake venom and spider toxin, and the soluble toxins of *Bact. Welchii* (gas gangrene) and of *B. anthracis symptomatici* (blackleg in cattle).

The antimicrobial sera constitute the majority of serum products. Included among these are antimeningococcic, antistreptococcic, antigonococcic, antistaphylococcic, antityphoid, antidysenteric, antirabic, antipneumococcic, antituberculosis, antiplague, anticholera, antihog cholera, antianthrax sera and sera for swine erysipelas, fowl cholera, white scours of calves, sheeppox, foot-and-mouth disease, canine distemper, rinderpest and spotted fever. The action of this group is directed more especially against the specific microörganisms involved, resulting in dissolution of the cells or lysis due to lytic bodies in the antisera (bacteriolysins).

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*Prepared by W. E. King.

In addition to the presence of lysins in antimicrobial sera, other antisubstances are known to exist, as agglutinins, bacteriotropins (opsonins), and precipitins. The antibody content of antimicrobial sera is comparatively little understood and the clinical interpretation of lysins, agglutinins and precipitins is not clear.

ANTITOXIC SERA

DIPHTHERIA ANTITOXIN.—A culture of the organism may readily be secured from the throat of a patient by transferring some of the diphtheritic exudate, on a sterile cotton swab, to Loeffler's blood-serum culture medium. After the growth of the bacteria at incubator temperature, contaminating organisms may conveniently be eliminated by the inoculation of a guinea-pig and the isolation of the diphtheria organisms from the tissues. A pure culture is necessary in the preparation of the antitoxin, but any given culture should not be relied upon until tests have been made of the final product.

To produce the diphtheria toxin with which the antitoxin horses are treated, the diphtheria organisms, in pure culture, are transferred to beef broth, contained in large flasks, and incubated at a temperature of 37°. A rapid growth takes place, during which the specific toxin is elaborated by the organisms. After a period of incubation of seven days, the bouillon culture is removed from the incubator, examined microscopically in order to make sure that contamination is not present, a preservative is added, usually carbolic acid, trikresol, or purified cresols, and the organisms are removed from the culture by passing the liquid through a Berkefeld filter. The filtrate (diphtheria toxin) is then placed in the refrigerator until used.

The horses which are used in the manufacture of antidiphtheritic serum, as well as for the preparation of other antisera, must be submitted to rigid inspection before being placed on the treatment. These animals, when purchased, are placed in a detention stable for several weeks. During this time they are subjected by a qualified veterinarian to a thorough physical examination and to the mallein test for glanders. Finally, only those animals which are pronounced normal in every way are admitted to the antitoxin stables. The stables and the operating rooms with their appointments, which are designed for the antitoxin horses, should be constructed with a view to perfect sanitation and cleanliness. Concrete floors, sanitary stalls, mangers, stocks and apparatus, good water, free ventilation and plenty of light should characterize the quarters.

The antitoxin horses are injected subcutaneously with the diphtheria toxin. The initial dose of toxin usually consists of only a fraction of a cubic centimeter, then increasingly larger doses are administered until the animals are finally able to receive 300 c.c. or more at a single treatment. The intervals between injections and the rate of increase of succeeding doses at any given time depend upon the condition of the animal. During this treatment a constant process of antitoxin formation is taking place in the body of the horse. In order to produce a potent serum, the injection of the toxin should be continued throughout the course of treatment as rapidly as the resulting reactions, following each injection of the animal, will allow.

After the completion of the initial toxin treatment, which occupies a period of from six weeks to three months, the horse is allowed a rest of about two weeks, during which time all the toxin which has been injected should be absorbed. During the remainder of the antitoxin-producing period of the animal's life (approximately two years), treatment with diphtheria toxin is continued at regular intervals. When desired, a small sample of blood serum may be secured from the horse for preliminary potency tests. Finally, the animal is bled from the jugular vein, under aseptic conditions. As much blood is secured as the horse can conveniently yield, varying in quantity from 10 to 15 l. The blood may be drawn through a sterile canula and rubber tube into tall, sterile glass cylinders. After the blood has clotted the serum separates and at the end of twenty-four to forty-eight hours, the clear, amber-colored fluid is poured from the cylinders into large, sterile glass containers, a preservative is added and the material is transferred to the laboratory. The serum is then passed through a Berkefeld filter.

Each lot of antidiphtheritic serum is submitted to rigid tests relative to potency, safety and microbial contamination. In determining the potency, varying amounts of the serum under test are mixed with the L+ dose of diphtheria toxin and injected into a series of guinea-pigs, each weighing 250 g.* The L+ dose of toxin is the least amount of toxin, which, when mixed with one unit of standard antitoxin (supplied by the Hygienic Laboratory) and injected into a guinea-pig of 250 g. weight, is sufficient to kill the animal in four days. From the results of this test it is possible to determine the smallest amount of the antitoxin which will protect a guinea-pig of 250 g. weight, when the animal has received simultaneously the L+ dose of toxin. This minimum amount of antitoxin represents one unit. Thus, if 1/500 c.c. of the given antitoxin represents the smallest amount which is capable of neutralizing the L+ dose of toxin, then the antitoxin would possess a potency of 500 units per c.c.

In order that the antitoxin may be tested for safety, each of several guinea-pigs is injected subcutaneously with about 2 c.c. of the serum. These animals are not released until the observer is satisfied that the serum contains no injurious properties. For the purpose of detection of microbial contamination, relatively large amounts of the antitoxin are placed in culture media and incubated under both aerobic and anaerobic conditions.

Diphtheria antitoxin is usually distributed in glass syringe containers ready for immediate use. After the product has been tested relative to potency, safety and microbial contamination, it is put up in sterile glass cylinders. These cylinders are so constructed that accompanying sterilized needles and pistons may be conveniently applied and the antitoxin injected hypodermically directly from the containers. Each container must bear a label indicating the number of antitoxin units enclosed and the date of preparation.

Finally, after the diphtheria antitoxin has been distributed in the glass cylinders, sealed and packed ready for use, sample packages are opened and examined for contamination, usually by two microbiologists. The product is not approved until the independent results of these final tests are compared, and it is assured that microbial contamination is absent.

All antitoxic sera should be kept away from the light and at a temperature of 10° to 15° whenever possible, as the presence of heat and light causes gradual deterioration. Usually an expiration date of from eighteen months to two years is applied to diphtheria antitoxin.

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* See Bulletin No. 21, Hygienic Laboratory, Washington, D. C.

It has been demonstrated that the antitoxic content of serum is closely associated with the globulins. Advantage is taken of this fact by most laboratories in reducing the volume of antitoxin, or concentrating the product, by precipitating the globulins with ammonium sulphate, redissolving the precipitate and dialyzing. The concentration of serum by this method increases the unit value per volume and tends to decrease the occurrence of undesirable secondary effects ("serum sickness").

TETANUS ANTITOXIN.—The processes involved in the preparation of antitetanic serum differ but little from those employed in the manufacture of diphtheria antitoxin. The pure culture of *B. tetani* is inoculated into large flasks of glucose bouillon, placed under anaerobic conditions and incubated at body temperature. A convenient method of excluding free oxygen, in the presence of which the tetanus organisms will not multiply, consists in boiling the glucose bouillon before the inoculation, to drive off the oxygen, then covering the liquid medium by a layer of oil. These cultures are subjected to a temperature of 37° for about two weeks, after which they are examined microscopically, preservative is added and the organisms are removed by filtration. On account of the presence of spores and the danger attending the contamination of any materials or biological products with the tetanus bacillus, it is important that great care should be exercised in the filtration and preparation of the tetanus toxin. Therefore, the filtration process is best accomplished in an isolated room which is used only for the preparation of tetanus toxin.

Tetanus antitoxin is produced by the injection of horses with the specific toxin and the same general methods and precautions are observed as in the preparation of diphtheria antitoxin. The antitetanic serum is tested relative to potency, safety and freedom from microbial contamination. *The standard unit of tetanus antitoxin is regarded as ten times the least quantity of antitetanic serum necessary to save the life of a 350-g. guinea-pig for ninety-six hours, against the official dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health Service.

Tetanus antitoxin is put up for use in the same manner as diphtheria antitoxin, being usually distributed in glass syringe containers. The product is used in both human and veterinary practice.

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* See U. S. Treasury Department, Public Health Reports, Vol. XXIV, No. 20, 1904.

Perfringens Antitoxin.—During the last two years of the recent world war, considerable attention was devoted to the experimental development of perfringens antitoxin or anti- gas gangrene serum. The use of this serum in conjunction with tetanus antitoxin was adopted by the War Department during the later months of the war. It is prepared by the injection of horses with *Bact. Welchii* toxin. The strength of the toxin is determined by the injection of pigeons which are uniformly susceptible to the substance. The antitoxin is standardized by injection of pigeons with mixtures of serum under test and varying amounts of standardized toxin. The sudden termination of the war did not permit the accumulation of conclusive evidence regarding the efficacy of perfringens antitoxin.

Antimicrobial Sera

In addition to diphtheria and tetanus antitoxins, certain other antisera are rapidly attaining practical significance. At present, however, no methods are in use by which any antisera other than diphtheria and tetanus antitoxins can be accurately standardized as to potency. Nevertheless, most of the products can be submitted to rigid tests in order to determine the presence of protective qualities.

Antimeningococcic Serum.—Horses are immunized to cultures of a number of strains of *M. intracellularis var. meningitidis*, the activity of the resulting serum being determined by agglutination and complement fixation tests. Antimeningococcic serum is used in the active treatment of cerebrospinal meningitis and is administered by lumbar puncture. The dose depends principally upon the age of the patient and the condition of the blood pressure.

Antistreptococcic Serum.—Bouillon cultures of *Strept. pyogenes* are killed by heating, and injected into horses in increasingly larger doses. Frequently, the killed cultures used in treating the horses are composed of several different strains of the streptococcus. In this case the resulting antistreptococcic serum is designated as "polyvalent," while the serum obtained after the injection of cultures consisting of but one strain of the organism, is called "monovalent" antistreptococcic serum.

In procuring the serum, handling, filtering, preserving and distributing for use, the methods are practically the same as those employed in the preparation of antidiphtheritic serum.

Antistreptococcic serum is carefully tested in regard to safety and freedom from microbial contamination. There are no methods available for definitely standardizing the product. The serum is often efficacious in cases of streptococcic infection.

ANTIGONOCOCCIC SERUM.—Killed cultures of *M. gonorrhœæ* are injected intraperitoneally or intravenously into large, healthy rams, or other animals. The dosage is increased and finally live cultures are applied, the degree of immunity acquired being determined by complement fixation and agglutination tests of the sera from the animals.

ANTIPNEUMOCOCCIC SERUM.—This is prepared by the injection of horses with pneumococci, type 1, and has been found to possess therapeutic properties when the disease is due to *Diplococcus pneumoniæ*, type 1. Antipneumococcus serum prepared from types 2 and 3 has been found to be of little value as compared with serum representing pneumococcus type 1. In using antipneumococcic serum from type 1 organism, it is therefore desirable, whenever possible, to isolate and type the pneumococci present in a given case under treatment.

DORSET-NILES (ANTI-HOG CHOLERA) SERUM (HYPERIMMUNE SERUM)*.—This product has been described in the preceding chapter under "hog cholera vaccine" (Double Treatment). When the hyperimmune serum is used unaccompanied by the virus, either among healthy or diseased swine, the process is known as the "Serum-Alone Method." Reichel† has succeeded in producing an antihog cholera serum which is sterile and free from inert solid matter by precipitating the globulins. Dorset and Henley‡ have announced the production of a clear and sterile serum by employing an extract of common garden beans together with salt to agglutinate the blood corpuscles.

ANTIRABIC SERUM.—Animals which have been immunized to rabies are bled and the immune serum may be used as a preventive and therapeutic agent. While this product is not often employed in practice, yet it has been shown by various investigators that considerable protection is obtained from its use.

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* See U. S. Bureau of Animal Industry, Bull. No. 102.

† Reichel, Proc. 18th Ann. Mtg. U.S.L.S.S. Asso., 1915, p. 127.

‡ Dorset and Henley, Jour. Agr. Research, Vol. 6, May 29, 1916.

ANTIDYSENTERIC SERUM.—Experimental monovalent and polyvalent antisera for epidemic dysentery have been developed by Shiga and Flexner, by the injection of horses with the filtrates from bouillon cultures of the dysentery bacillus.

THE PRESERVATION OF ANTISERA.—The question of a proper preservative for antisera has received much attention. The problem of preservation involves several conditions, as the ideal preservative, when incorporated in the proper volume of serum in efficient dilutions, must possess marked inhibitive and germicidal power, it must prove inert when injected into the patient, and it must produce no objectionable precipitation of serum proteins. At present, trikresol or purified cresols (0.4 of 1 per cent) is generally employed.

BIOLOGICAL PRODUCTS OTHER THAN VACCINES AND ANTISERA

TUBERCULINS.—*Koch's Tuberculin* (*Old*).—Koch's tuberculin is the concentrated, glycerinated, beef bouillon in which *Bact. tuberculosis* has been grown. The active substance of the tuberculin is apparently an albuminous body insoluble in alcohol. The product is harmless for the non-infected, but exerts a toxic action upon tuberculous individuals, the reaction being characterized by a rise in temperature which begins two to ten hours after treatment, continues for a few hours and finally subsides. Tuberculin (old) is used as a diagnostic agent in both human and veterinary practice.

Tuberculin (old) is prepared from cultures of the human or bovine variety of *Bact. tuberculosis*. Apparently the active product can be obtained from attenuated as well as from virulent cultures. The organism is inoculated into beef bouillon to which 5 per cent glycerin has been added. The culture medium is usually distributed in flasks and the tubercle organisms, when inoculated, are carefully placed on the surface of the medium. The cultures are incubated at a temperature of 37° to 38° for six to ten weeks, during which time a heavy growth slowly spreads over the surface of the medium and finally falls to the bottom of the flasks. In the successful preparation of tuberculin it is important that the cultures should remain undisturbed, having access to plenty of air, that the incubator temperature should be constantly maintained without fluctuations, and that the organisms should be allowed to grow until they have completely elaborated the active "tuberculinic" substance. After the growth is complete, the cultures are removed from the incubator and sterilized in streaming steam. The killed cultures are then evaporated over a water bath to one-tenth the original volume, the bacteria are removed by passing the cultures through paper and Berkefeld filter and a preservative is added. For cattle the dose of tuberculin concentrated by evaporation to one-tenth the original volume, is 0.25 c.c. to 0.7 c.c. Because of the fact that the material is thick and syrupy in consistency and the dose is inconveniently small, it is usually diluted with seven parts of weak carbolic acid solution. During the preparation it may be evaporated to four-fifths the original volume and preserved by the addition of 1 per cent. carbolic acid of sufficient volume to dilute properly. The United States Department of Agriculture, Bureau of Animal Industry, requires that a dose of tuberculin for the subcutaneous test of cattle shall not be less than the equivalent of 0.5 g. of Koch's Old Tuberculin. According to this requirement the dosage for cattle must depend upon the amount of Koch's Old Tuberculin contained in any given brand of the product. The dose of tuberculin prepared by most laboratories should not be less than 5 c.c. as each c.c. usually contains the equivalent of 0.1 g. Koch's Old Tuberculin. The product should be tested for activity by injecting known tuberculous animals with the tuberculin under test. The presence of typical reactions in tuberculous animals indicates the reliability of the product. Tuberculin may be subjected to experimental test for activity by the method suggested by the Bureau of Animal Industry, the essentials of which are as follows:

A series of guinea-pigs are inoculated with a suspension of fresh tubercular material taken from lesions in tubercular cattle. After the development of tuberculosis in the guinea-pigs a second series of guinea-pigs may be injected with a suspension of tubercles from the first series of infected guinea-pigs. These animals should be weighed at regular intervals and carefully examined for evidences of tuberculosis. After a period usually of about three or four weeks, a series of guinea-pigs should be selected which show unmistakable evidence of tuberculosis. This series together with controls may be injected with varying doses of tuberculin under test, in order to determine the degree of sensitiveness to the lot of tuberculin in question. One group of tubercular guinea-pigs should be injected with Bureau of Animal Industry Tuberculin of known potency which serves as a standard. The dose of tuberculin to be injected in the test guinea-pigs should be the equivalent of 0.25 g. of Koch's Old Tuberculin per 500 g. weight guinea-pig.

Conclusions may be drawn from such tests according to the following suggestions made by the Bureau of Animal Industry:

If the tuberculin which is provisionally taken as the standard kills not less than two-thirds of the sensitized guinea-pigs injected with it before the lapse of twenty-four hours, and the two normal guinea-pigs injected with it remain free from symptoms of disease excepting the rapidly passing distress which may immediately follow the injection, it is required that any other sample of tuberculin, if it possesses a reliable degree of potency, should kill, within twenty-four hours, at least half the sensitized guinea-pigs injected with it, and that the normal guinea-pigs injected with it should be alive and well at the end of twenty-four hours.

In human, as well as in veterinary practice, tuberculin may be applied as a diagnostic agent in various ways. In addition to the hypodermic injection of tuberculin (old), as described above, the method of Calmette,* von Pirquet† and Moro‡ may be used in human practice. Calmette's ophthalmo test consists in the instillation in the eye of Koch's purified or refined tuberculin. Purified tuberculin is prepared by treating the original tuberculin with absolute alcohol, washing and drying the precipitate. One drop of a 1 per cent solution of purified tuberculin is placed in the eye. A positive reaction is manifested by a congestion of the palpebral and ocular conjunctiva a few hours after the application of the tuberculin. The method of von Pirquet* depends upon the cutaneous application of the tuberculin. One drop of tuberculin (old) is placed on the arm, after cleansing the skin, and the small area under the drop is scarified. Two or more small areas may be treated in this way, as well as a control area treated with sterile salt solution or a solution of glycerin and dilute carbolic acid in substitution for the tuberculin. The appearance of a reddish zone in from twelve to twenty-four hours indicates a positive reaction. This area of inflammation gradually increases somewhat in elevation and diameter and finally subsides in a few days. Moro's modification of von Pirquet's method consists in the use of tuberculin ointment prepared by the combination of tuberculin (old) and anhydrous lanolin in equal parts.

* Calmette, Presse Medicale, 1907, 15.

† von Pirquet, Berl. klin. Woch., 1907, 44.

‡ Moro, Münch. med. Wch., 1908.

The ointment is vigorously rubbed on a small portion of the skin of the abdomen. A positive reaction is evidenced by the appearance of a distinct granular or papular eruption at the point of application after about twenty-four hours.

For the diagnosis of tuberculosis in cattle, the intradermal test is generally regarded as next in importance to the older subcutaneous test. In conducting this test 0.1 to 0.3 c.c. of a 50 per cent solution of tuberculin is injected into the cuticle layer of the skin at the base of the tail. A positive reaction is present when, twenty-four to seventy-two hours after the injection of tuberculin, the localized area of skin shows a circumscribed œdematous swelling.

Tuberculin (old) is usually distributed in small vials, sealed and labeled. The labels should indicate the amount and dosage and the date of preparation. Under the influence of light and heat the fluid product may slowly deteriorate; therefore, when possible, it should be kept in the refrigerator until needed.

Other Tuberculins.—Koch introduced tuberculin "T. R." (tuberculin residuum) in 1897 and tuberculin "B. E." (bacillary emulsion) in 1901. The former is prepared by repeatedly centrifugalizing a suspension of the dried and ground tubercle organisms in water. The supernatant fluid "T. O." after the first centrifugalization is discarded and the final product consists of the constituents of the bacteria which are insoluble in water. One cubic centimeter of the tuberculin "T. R." should contain the equivalent of 2 mg. of the dry tubercle solids. Tuberculin B. E. is composed of a suspension of crushed or thoroughly ground tubercle organisms in 50 per cent glycerin solution. Each cubic centimeter should contain the equivalent of 5 mg. of tubercle solids. Tuberculin T. R. and tuberculin B. E. are used as therapeutic agents, the latter probably being regarded with more favor by clinicians. The material is administered by subcutaneous injection, the time intervening between successive treatments varying from three to ten days. The initial dose recommended by most investigators, is 0.0001 mg. or less.

MALLEIN.—Mallein is prepared from cultures of *Bact. mallei* by practically the same methods as those employed in manufacturing tuberculin from *Bact. tuberculosis*. The product is used for the diagnosis of glanders. A few hours after mallein is injected, subcutaneously, into glandered horses a severe local reaction and a rise of temperature usually follow. The thermal reaction is very similar to that produced in tuberculous animals by the injection of tuberculin. The local swelling caused by mallein treatment is considered by some to be quite as diagnostic as the temperature reaction.

The ophthalmic mallein test, a comparatively recent method, which was first used by Choromansky, appears to be attaining considerable recognition as a valuable aid in diagnosis. The test consists in the application of concentrated mallein to the inner canthus of the eye. A drop of the concentrated mallein in liquid form or a small bit of the same in desiccated condition may be used. In a positive case, hyperemia and swelling of the conjunctiva and a purulent exudate at the inner canthus of the eye will appear from four to six hours after the instillation of the mallein.

Goodall* advocates the use of the intrapalpebral mallein test which involves the injection of a small dose of mallein under the skin of the eyelid.

The stock culture of the glanders organism used in the preparation of mallein should be one which possesses known virulent properties. It is grown at a temperature of 37° for three months in flasks of glycerin bouillon having a chemical reaction of about three points acid to phenolphthalein. When the cultures are removed from the incubator they are heated in streaming steam, passed through a Berkefeld filter and the filtrate is concentrated, preserved and distributed in labeled vials.

Suspensions for the Agglutination Tests

Agglutinins are hypothetical bodies existing in the blood and possibly other body tissues, of an individual affected with, or convalescent from, a specific infectious disease. The bodies possess the power of "clumping" and precipitating the specific bacteria which are the cause of the disease in question. Thus, if a dilution of blood serum from a typhoid fever patient is mixed with living typhoid organisms, the specific agglutinins present in the serum will cause the organisms to cease their motion and agglutinate or clump together in irregular masses. Normal human blood serum placed under the same conditions will fail to cause the agglutination of the organisms in similar dilutions. The agglutination reaction may, therefore, be used in the diagnosis of certain specific infectious diseases. The serum must be properly diluted in order that the reaction may be of diagnostic value, because undiluted, normal serum will cause a positive agglutination reaction in most cases.

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* Goodall, Jour. Comp. Path. and Therap. 1915, Vol. 28, p. 281.

The agglutination test is used as a practical aid chiefly in typhoid fever in man and glanders in horses. The test may be conducted either microscopically or macroscopically. In the microscopic method, the diluted serum from the suspected case is placed under the microscope with the live, specific organisms in hanging drop. In the macroscopic method, the serum is added to an emulsion of the killed (heated) bacteria in small test-tubes, and the resulting reaction detected with the naked eye.

The emulsion, suspension or "test fluid" for the typhoid agglutination test is prepared from a pure culture of *B. typhosus.* The organism is grown for twenty-four hours upon agar at a temperature of 37°. The growth is then removed from the surface of the agar, placed in sterile, physiologic salt solution and the organisms killed by heating on a water bath at a temperature of 60° for one-half hour. The emulsion is then roughly standardized by adding sufficient sterile, physiologic salt solution to impart to the fluid the required degree of cloudiness, when compared with control emulsions. To the suspension of dead typhoid organisms or "test fluid" a preservative, usually formalin, is added and the product is distributed in properly labeled bottles. In conducting the test, the suspected typhoid serum is placed in small tubes, each containing 1 c.c. of the suspension fluid, in such proportions that the serum is diluted 1:50, 1:100, and 1:200. A flocculent precipitate of the dead organisms indicates a positive reaction.

Suspension fluid for the glanders agglutination test is prepared in practically the same manner as the typhoid test fluid. The glanders organisms are grown on acid agar and the suspension fluid is usually preserved by the addition of carbolic acid. In conducting the glanders agglutination test, the suspected serum is usually placed in the following dilutions: 1:200, 1:500, 1:800, 1:1200, and 1:1800.

The agglutination reaction has been applied experimentally and practically, with more or less success, in the diagnosis of Malta fever, Asiatic cholera, bubonic plague, pneumonia, tuberculosis, contagious abortion (bovine) and other infectious diseases.

SUBSTANCES USED FOR DIAGNOSTIC TESTS

LUETIN.—Noguchi* has developed a preparation known as *luetin* which is used in the diagnosis of syphilis. The material is prepared from a number of strains of *Spirochæta pallida* grown, under anaerobic conditions, on special ascites agar and bouillon media. After abundant growth of the spirochetes occurs, the agar cultures are ground and mixed into a paste. To this material fluid cultures are added in sufficient proportion to form a liquid emulsion. The organisms are then killed by heating at 60° for one hour and a preservative is added.

In applying this diagnostic material to a suspected syphilitic case 0.05 c.c. is very carefully injected into, not beneath, the skin. An area on the antero-internal surface of the upper arm is usually chosen as the site of injection. A positive diagnosis of syphilis is indicated if, after the third day a marked cutaneous eruption appears at the point of inoculation.

ANTIGENS.—Certain antigens, such as gonococcus and syphilitic antigen, are of value for the purpose of conducting complement fixation tests in laboratory diagnosis. Gonococcus antigen consists of an extract or filtrate prepared from a suspension of polyvalent gonococci. Syphilitic antigen consists of an extract prepared from either luetic or certain normal tissues such as beef or human heart muscle. Tuberculosis antigen, as described by Craig,† consists of the filtrates of specially prepared cultures of *Bact. tuberculosis*.

THE SCHICK TEST.—The susceptibility or non-susceptibility of individuals to diphtheria may be determined by the application of the test described by Schick.‡ For this purpose standardized diphtheria toxin is required. 0.1–0.2 c.c. of a relatively fresh normal saline solution containing 1/50 minimum lethal dose of diphtheria toxin, for a 250-g. guinea-pig, is injected intracutaneously. The appearance of a circumscribed area of redness at the site of injection after twenty-four to forty-eight hours indicates that the individual possesses practically no immunity against diphtheria.

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* Noguchi, H.: Jour. Exp. Med., xiv, Vol. 16.

† Craig, Am. Jour. Med. Sci., 1915. 150, p. 781.

‡ Schick, Munch. Med. Woch. 1913, 60, p. 2608.

CHAPTER V*

CONTROL OF INFECTIOUS DISEASES

PRINCIPLES

That the infectious diseases can be controlled depends upon the facts that they arise only in the presence of a specific living infective agent; that they pass from patient to prospective patient only because the infective agent passes from patient to prospective patient; and that therefore the prevention of effective passage will prevent the spread of the disease. These preventive measures with their natural incidental developments constitute the practice of present public health relating to these diseases.

In general the infective agent leaves the body of the patient by the mucus-lined orifices of the body, the nose and the mouth, the anus, the urethra, the mammæ, and the genital organs. In general it must, if it is to infect successfully another person, reach one or more of the same mucus-lined orifices of that other person. Excluding the venereal diseases the ordinary infectious diseases (tuberculosis, typhoid fever, diphtheria, scarlet fever, measles, whooping cough, smallpox, chickenpox, pneumonic plague, leprosy) are received almost exclusively into the body through the mouth (or nose). While the passage is usually from mucous membrane to mucous membrane as above outlined, the infective agent may pass effectively from mucous membrane to cut or abrased skin (the uninjured skin is probably almost always resistant to these infections). Again, in those diseases where skin lesions are a prominent feature (smallpox, plague, leprosy) the infective agent may pass from the skin lesions to a mucous membrane, or to a cut or abrasion. But these are rare methods of transmission as compared with the mucous to mucous forms, except in syphilis and chancroid where they frequently occur.

The routes of travel between the patient and the prospective patient are many. At times, mucous membrane may be applied to mucous membrane as when a well person kisses a diphtheritic child; conveyance of particles through the air, sprayed from the mouth, may occur, as when a diphtheritic patient coughs into an attendant's face; or mucous membranes may be applied to skin or *vice versa*, as in the kissing of a smallpox patient; but in general the discharges are conveyed somewhat indirectly. The prime route from mucous membrane to mucous membrane is furnished by the hands. An attendant touches the patient's lip or wipes out the mouth or otherwise performs toilet services and receives the discharges upon his fingers. The fingers go then to the attendant's mouth directly, or touch something (the tines of a fork or the bowl of a spoon, etc.) which in turn goes into his mouth; or the attendant may touch the fork or spoon or food of others and thus they become infected. He may milk a cow and so get the discharges into the milk. With the infection in his own mouth he may kiss others and transfer it to them. It is impossible to outline the infinite combinations that may occur, but the principles are here made obvious. When the infective discharges handled are those of the bladder or bowel (as in typhoid fever, cholera, etc.) the same dangers of transmission are encountered and unfortunately too often realized. The wholesale discharge of sewage into water supplies is merely a gross example of the same principle of transfer of discharges from human bodies to the human mouth.

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* Prepared by H. W. Hill.

Another factor in the transmission of disease (as distinguished from the transmission of the germ) is the condition of the infectee. The germ is analogous to a seed; the methods of transmission are somewhat analogous to the distribution of seeds in nature; the condition of the infectee is analogous to the character and nutritional condition of the soil which the seed reaches.

If for any reason the germ will not develop in the soil where it is planted, or, still further, if it grows but fails to produce those poisons through which alone it acts, or finally if, growing and producing its poisons, the soil neutralizes the poisons, no disease results. Science, logic, and the law (each of which regards itself, and rightly so, as merely an apotheosis in its own line of "common sense") unite in the dictum that a disease exists only when the normal functions of the body are in some way interfered with to the detriment of the body. The mere infection of the body with a disease germ does not, in science, logic, or the law, constitute disease. Hence, the reception of a disease germ into the body is but the first of three essentials, the other two being poison-production by the germ and poison-action on the tissues. Many persons are insusceptible to the poisons of one or more disease germs. In whatever way this insusceptibility originate, (natural, acquired by a previous attack, or acquired by artificial treatment) the existence of insusceptibilty tends to prevent the acquiring of the disease.

PRACTICE

Undoubtedly, the one wholly efficient method of preventing the spread of infectious diseases would consist in immunizing all the possible infectees against all the possible diseases. Unfortunately, we know of no practical immunizing methods except in the case of a very few diseases, notably smallpox and typhoid fever, paratyphoid and cholera.

Our methods of control of any disease therefore begin with the attempt to destroy them at their origin in the body of the patient, but such methods are merely incidental to the destruction of the germs for the good of the patient himself, *i.e.*, they belong rather to therapeusis than to public health. Unfortunately, also, scarcely any efficient method of destroying bacteria within the body of the patient without destroying the patient also is known and therapeusis along this line contents itself largely as yet in so controlling the patient's condition as to permit and encourage to the highest the natural forces of the body to attack the germs. These natural forces, however, direct their chief energies and secure their chief results, not in destroying the germ but in neutralizing the poisons the germs throw off, and in practice, patients recover rather because they have neutralized the poisons than because they have killed or ejected the germs. For this reason a recovered patient often remains a breeding ground for the germs which caused the attack, but to whose poisons he is now resistant or immune.

Practically, then, the germs must leave the patient's body before they can be destroyed. It is at this stage that the most efficient control can be exercised, and that control consists in killing them before they become scattered. In practice the efficient disinfection of all the discharges of a patient will prevent the spread of any disease from him. But this is not as easy to do as at first might appear. Ridding the body of its discharges in health is a process dependent on the individual, carried out by him consciously or unconsciously all his life by methods chiefly directed to conserve convenience rather than to prevent their spread. In health, the careless scattering of these discharges is not of great moment, but of course the habits of indifferent and careless discharge, acquired in health, persist after disease is contracted. The presence of an infective agent in the discharges renders the previously harmless scattering of the discharges the greatest menace that is known to the health of the associates. Hence one primary requisite in the personal warfare against the infectious diseases is to establish among all people such habits during health that even the normal discharges are not exchanged. This must be achieved by teaching the individual not to scatter his discharges and by teaching his associates not to receive them, if he does.

Accepting conditions as they are, the care of the sick by watchful, well-trained nurses who will prevent the spread of the discharges must largely take the place of the earlier training of the patient. Usually this also is impossible. It would seem that at least 95 per cent of the total cases of infectious disease in this country are cared for at home by the home folks, *i.e.*, untrained, worried, exhausted mothers chiefly, trying to learn in the actual face of the enemy, the technic and knowledge acquired quietly and systematically by the trained nurse. Hence, within the home, and at present, sanitary nursing to prevent spread of disease is a poor and often broken defence.

The third method of control is the destruction of the germs in passage from patient to prospective patient; and this must be largely confined to the actual discharges when accumulated in one place; the finer discharges thrown into the air can hardly be followed.

Under this head may be classed the disinfection of fæces and urine, the disinfection of bed clothing, eating utensils, etc., coming into contact with the patient, and especially the disinfection of the hands of attendants. The throats of attendants often contain the germ, especially when diphtheria, scarlet fever, measles, etc., are concerned. Unfortunately, the disinfection of the throat is extremely difficult and the scientific nurse will take every precaution to avoid receiving the germ into the mouth, rather than try to dislodge or destroy it after its reception. A respirator is useful for this purpose.

As outlined in the preceding section, the principles involved in controlling infectious diseases are very simple, but in practice the individual cannot be trusted to avoid spreading his discharges, partly from ignorance, partly from carelessness, often from mere ingrained bad habits regarding the disposal of discharges, especially those of nose and mouth, indulged unconsciously by those who both know how and mean to be careful.

This would matter little were the infected persons always so sick as to be confined to the house or to bed, especially if during such confinement their discharges were under strict surveillance by scientific trained nurses.

But since many, perhaps half, of the infected persons are not sick enough (if sick at all) even to remain at home; since, also, even severe cases, under surveillance in bed during the height of the attack, have a prodromal stage and a convalescent stage during which they are going about although infective, it is not hard to see that the population of any community is likely to embrace at any time infective persons at large—persons who may or may not be aware of their own condition.

Theoretically and practically, then, the official control of infectious diseases must begin with the blanket assumption that the discharges of every individual must be confined to himself and especially prevented from reaching, through any public utility, the mouths of other citizens. Official control of the exchange by individuals of discharges within the family and in the absence of any specific proof that the discharges are infective, is impossible, although through various agencies the individual may be urged to that end. The moment, however, that the individual or the family engage in any occupation which permits them to inflict their discharges upon others, especially through food or milk, that moment should the individual or family come under official cognizance, their methods be inspected and their infectiveness estimated. The same arguments apply to aggregations of individuals from different families. So long as private meetings are held, it is difficult to supervise or prevent exchange of discharges. But public and especially compulsory meetings, at school, at church, at theatre, etc., should receive official attention. Provision should be made concerning all such meetings that they be held only in suitable places, without overcrowding. The exclusion by the officers, attendants, or the general public of all known to be infected or suspected of infection and of all who more openly disregard ordinary rules of decency in the disposal of discharges (spitting, etc.), should be part of the duties of the health department.

Finally, the strictest supervision of those concerned publicly and officially in the handling of public utilities on a large scale (water supplies, milk supplies, hotels, restaurants, food stores, etc.) should hold all strictly accountable for the contamination of such supplies with discharges whether these be normal or not. Hence official control of infectious disease divides itself naturally as follows:

1. The recognition and isolation of frank cases of the diseases in question, at home or, better, in a proper hospital.

2. The supervision of the attendants and immediate associates of such frank cases.

(*a*) To detect among them that one from whom the frank case, already recognized, received his infection.

(*b*) To detect at the earliest moment any other frank case about to develop from among those associates who may have been infected at the same time and from the same source as the frank case already found.

(*c*) To prevent further spread from any already infected associates or those who may become infected by later association with the frank case during its existence as such.

(*d*) To discover mild, unrecognized, and concealed cases, and carriers.

3. The exclusion of all infected persons, their infected non-immune attendants, and immediate associates, from participation in public life so long as danger continues and especially their exclusion from having to do with public utilities or public gatherings. Hence has arisen the crude drastic but efficient (when consistently and uniformly carried out in every case) system of isolation of the sick and quarantine of his associates.

Unfortunately quarantine has become a mere letter-of-the-law procedure, working great hardships on those who conscientiously submit to it and yet failing to achieve its objects because of the great number of those who evade or escape it; moreover, because its provisions are unintelligently enforced. Of what avail is rigid quarantine of an infected family where milk continues to be sold from the same premises? Why quarantine the honest man who has an honest physician and whose case is reported, while his neighbor, having the same disease in his family, calls no physician, or a dishonest one, and therefore escapes official cognizance?

The only remedy seems to be the recognition of the principle that harboring or having in possession a case of infectious disease, unknown to the proper officials, is a crime against society, and that the excuse that the person harboring such case did not know it to be such should be of no more weight than the plea of ignorance of the law which is not allowed in other and often far less serious matters.

The official isolation of infectious cases involves also official responsibilities regarding the release from isolation after the acute attack is over. Officially to declare a person dangerous to the community does no harm to the community if a mistake is made. An official declaration that a person is no longer dangerous and is therefore free to enter into the community life again may, if mistaken, result in a widespread outbreak. No more delicate task confronts the public health official than the making of this decision.

In diphtheria, the examination of cultures from the throat and nose of the person in question and the repeated failure to find the bacterium of diphtheria is usually considered a safe criterion. In scarlet fever, complete and continued restoration of the throat and nose to normal conditions, together with absence of ear discharges, should be required, yet is not perfect; for it is not very unlikely that the scarlet fever infective agent, whatever it may be, can continue in a recovered scarlet fever throat as the diphtheria bacterium may remain in a recovered diphtheria throat. In other diseases the decision is based on similar lines—the disappearance of crusts in smallpox and chickenpox, of discharges in measles, on restoration to normal of whooping cough; but in all these diseases the analogy with diphtheria may hold to a greater or less extent. In tuberculosis, the patient is infective as long as *Bact. tuberculosis* can be found in the sputum; in typhoid fever the patient is likewise infective as long as the urine or fæces show the typhoid bacillus. In these two diseases, however, quarantine or even isolation is not officially carried out nor release from restriction officially given to any great extent or with any marked uniformity.

Full sanitary nursing precautions regarding a typhoid fever patient's discharges should continue for an average of three months after recovery.

PUBLIC HEALTH METHODS

By request of the editor, there are here inserted the rules followed in the isolation and quarantine of the ordinary infectious diseases in London, Canada, where the writer was Medical Officer of Health, 1915–18; Captain and Sanitary Officer to the 1st Military District, Canadian Expeditionary Force, 1916–18; and Director of the Institute of Public Health since 1912.

PUBLIC HEALTH METHODS, LONDON, CANADA, AS REVISED AND PROMULGATED BY THE INSTITUTE OF PUBLIC HEALTH OF WESTERN UNIVERSITY, LONDON, CANADA

Public Health regulations are made for the good of the Public. They are as lenient as possible, consistent with the prevention of disease. They are not made to interfere with the individual's freedom, except as such freedom is dangerous to others.

I. HOUSEHOLDER'S RESPONSIBILITY TO THE BOARD OF HEALTH

1. Infectious Diseases:

It is required that whenever any householder knows or has reason to suspect that any member of the household has any communicable disease, he shall within twelve hours notify the Health Department.

NOTE.—The Medical Officer of Health has the right to enter any house, etc., in which he knows or has reason to suspect the presence of any communicable disease.

2. Births:

It is required that every birth (including stillbirths) shall be registered by the parent or guardian, in the prescribed form, within thirty days after the date of birth.

3. Deaths:

It is required that every death (including stillbirths) shall be registered by a member of the household in which the death occurs, in the prescribed form, before a burial permit is issued.

II. PHYSICIAN'S RESPONSIBILITY TO THE BOARD OF HEALTH

1. Infectious Diseases:

It is required that whenever any physician knows or has reason to suspect that anyone whom he is called upon to visit is infected with any communicable disease, he shall within twelve hours notify the Health Department.

2. Births:

It is required that every birth (including stillbirths) be registered by the physician in attendance, in the prescribed form, within thirty days after the date of the birth.

Note.—Further it should be the duty of every physician to see that every birth (including still-births) at which he is in attendance, is properly registered by the parent or guardian.

3. Deaths:

It is required by law that every death (including stillbirths) be registered, by the physician last in attendance, on the prescribed form, before a burial permit is issued.

Note.—Further it should be the duty of the physician to state the cause of death clearly and in accordance with the "International List of Causes of Death."

III. Penalties

1. Any person (householder or physician) whose responsibility it is, by the Statutes of Ontario, to report a case of any communicable disease, and who neglects to do so, may incur a penalty not less than $25.00 nor exceeding $100.00.

2. Any person required by the Statutes of Ontario to register a birth or death, and who neglects to do so, may incur a penalty not exceeding $10.00.

3. Any person who wilfully makes or causes to be made a false statement touching any of the particulars required in registering a birth or death may incur a penalty of $50.00.

3. Any person who wilfully makes or causes to be made a false statement touching any of the particulars required in registering a birth or death may incur a penalty of $50.00.

IV. List of Communicable Diseases which Must be Reported in Ontario

Smallpox
Leprosy
Scarlet Fever
Diphtheria
Bubonic Plague
Asiatic Cholera
Measles
Syphilis
Anterio Poliomyelitis (Infantile Paralysis)
Cerebro-spinal Meningitis
Typhoid Fever
Chickenpox
Whooping Cough
Mumps
Gonorrhea
German Measles
Glanders
Anthrax
Tuberculosis (of all forms)
Rabies (Hydrophobia)
Erysipelas
Any other communicable disease

V. Permits to Leave Quarantine, Etc.

1. The immune contacts and others, who under the schedule, may be allowed to attend business, school, etc., despite quarantine on other members of the household, MUST HAVE WRITTEN PERMITS from

the M. O. H. so to do. Recovered patients cannot return to school or business or otherwise escape quarantine without such permits.

2. The attending physician, while legally bound to report all infectious diseases, cannot legally impose or release quarantine. No one but the M. O. H. can legally impose or release quarantine.

VI. Methods of Handling the Infectious Diseases—London, Canada, January, 1916

1. Tuberculosis:

All cases in all stages are reportable within twelve hours of discovery, to the M. O. H. who is thereafter legally responsible for the prevention of spread of the disease.

In all non-infectious stages, this supervision is restricted to watching—through the attending physician, if there be one—the progress of the case in order to detect the development (if any) of an infectious stage.

There are no restrictions other than the above on the patient during non-infectious stages.

In all infectious stages, definite arrangements must be made to prevent infection: these may be made through the attending physician, if there be one; otherwise directly with the relatives or patient.

There are no restrictions on the associates of the patient. Therefore placarding and other quarantine measures are not necessarily imposed. Terminal disinfection, by Board of Health, is free in tuberculosis. It is not performed in other diseases, except under special circumstances.

2. Epidemic Anterior Poliomyelitis (Infantile Paralysis):

All cases are reportable in the acute stage. The patient must be isolated during the continuance of fever: and the usual precautions relating to disinfection of discharges must be carried out during the progress of the disease.

3. Epidemic Cerebro-spinal Meningitis:

As in poliomyelitis, with the addition that nurses, etc., shall wear respirators during the performance of intimate personal services to the patient.

4. Rabies:

Persons bitten by dogs suspected of Rabies should receive the Pasteur Treatment, furnished by the M. O. H. through the Provincial

Board of Health. Dogs who have bitten persons should not be killed but should be securely confined for observation. If surviving a week the diagnosis of not Rabies is established.

5. Venereal Diseases:

Under Provincial legislation passed in 1918, everyone (except lay householders) who is responsible under other laws to report other infectious diseases is required to report venereal diseases, but the patient's name is not to be given (serial numbers to be used instead) unless the patient abandons treatment, whereupon full identification data must be sent the Medical Officer of Health, who must then follow up the case and take proper methods for prevention of spread. The legislation is very detailed and complete. Military and civil coöperation is assured also.

6. The other Rarer, Infectious Diseases, which are reportable:

Anthrax, Asiatic Cholera, Bubonic Plague, Glanders, Leprosy, Erysipelas, etc., will be handled as they arise according to the circumstances of the case.

7. The More Common Infectious Diseases:

Smallpox, Scarlet Fever, Measles, Diphtheria, Typhoid Fever, Chickenpox, Whooping Cough, Mumps and German Measles are handled according to a schedule given beyond.

Free Diphtheria antitoxin, etc., is supplied on application to the Medical Officer of Health.

DEFINITIONS

"Cases" are persons, sick of the disease in question. Those exposed to an infected person, during an infectious stage, whether that infected person be sick (a "case") or well (a "carrier"), are known as "contacts." The contacts may be immune or not, infected or not. It is the fact of exposure to infection that makes them "contacts."

The term "isolation" means restriction of freedom of an internally infectious person, *i.e.*, of a person sick with the disease (a "case"), or of a person, whether immune or not, who is well but infectious, and therefore is a "carrier." A non-immune "carrier" may become a "case," if the disease develops in him later.

The term "quarantine" is properly applied only to restriction of freedom of non-immune well persons who have been so exposed to infected persons as to make it likely that they themselves may develop the disease. Neither isolation nor quarantine can be justified if imposed on non-infectious immunes, or on non-immunes who have not been exposed.

Rules for Release of "Cases" from Isolation

The case, whether isolated at a hospital, at home with a trained nurse in attendance, or at home without a nurse, will be set free, and may go to school, work, etc., only after full recovery as determined by the M. O. H., and in addition as follows:

In Smallpox, after all scales, plaques, crusts, etc., have disappeared, as determined by the M. O. H.

In Scarlet Fever, not less than six weeks from onset and then only if temperature, nose, throat, ears, etc., have been normal for one week, no discharge of any kind from any orifice exists, and all wounds, sores, herpes, etc., are completely healed.

In Measles proper, on the fourteenth day from onset, which will be the tenth day from beginning of rash.

In Diphtheria, after three consecutive negative cultures have been obtained from both nose and throat.

In Typhoid Fever, eight weeks after onset.

In Chickenpox, after all crusts, scales, scabs, etc., have disappeared.

In Whooping Cough, one week after last whoop, or six weeks from first whoop, whichever comes first.

In Mumps, three weeks from onset.

In German Measles, one week from onset.

Placarding of House—External

If the case goes to a hospital, no placard is placed on the home from which he came, notwithstanding that exposed non-immunes may be quarantined there, unless the quarantine is not properly observed; whereupon a placard may be used in any disease, or a special policeman detailed to watch the house or both.

If the case remains at home, where the family occupies a house, both front and rear entrances are placarded in certain diseases (Smallpox, Scarlet Fever, Measles, Diphtheria and the "Rarer Infectious Diseases" mentioned above, under the Provincial Law; in certain others, typhoid fever, chickenpox, whooping cough, mumps, German measles, placarding is optional and is enforced only if isolation or quarantine is evaded by the inmates. In hotels or apartment houses, only that room, suite or apartment occupied by the patient and his family need be placarded; otherwise the same rules should be followed.

Milk men are warned that they may continue delivery, but must take no bottle or other receptacle away until the placard is removed, and that all such receptacles must be sterilized before use.

Placarding of House—Internal

Under whatever circumstances isolation or quarantine is carried out in houses, hotels, apartments, or elsewhere, except in contagious hospitals, there is fixed to an internal wall at a convenient point a notice, giving the general rules regarding who are isolatable, who are quarantinable, who may go free, and the actual names of all inmates are distributed in writing into these three groups on the notice. For all who may go free, permits in writing are furnished and the fact that permits to go back and forth have been issued is also recorded on this notice.

We believe the internal placard, properly filled out as above, to be of the greatest educational value; it brings everything down to "brass tacks," inasmuch as the family is classified as to immunity or non-immunity, freedom or restriction, by name in writing upon it, and because there can therefore be no misunderstanding on the part of any individual concerned.

In the writer's opinion, an internal placard should be used in all infectious diseases to the exclusion usually of the external placard; the latter being used only when the rules laid down in the internal placard are broken wilfully by the inmates.

Quarantine Periods for Contacts

Contacts are those persons who come into such close relationships with an infected person, *during an infectious stage*, as may reasonably be held to afford opportunity for the transfer of the infection from the infected person to the other. The existence of such opportunity for infection by no means ensures its occurrence, but in most diseases there is no sure way of determining whether or not infection has occurred other than by watching to see if the disease develops. This period of observation is usually the period of quarantine. In diphtheria, contacts may be examined by taking a culture from nose and throat. If the culture prove negative the contact, whether immune or not, may be set free because, since he is not infected, he will not become sick himself nor can he give the disease to others. If it prove positive, the contact, whether immune or not, is dangerous, even though not sick, and should be isolated as dangerous; many physicians advocate giving a prophylactic dose of antitoxin (1000 units) to prevent the development of the disease in such contact. This dose ensures the non-development of the disease for two weeks, at which time it must be renewed if protection is still desired. Prophylactic doses, indeed, even therapeutic doses (10,000 to 50,000 units), do not affect the bacilli themselves, and therefore the infected contact who has been protected against the disease by antitoxin so far as his own health is concerned, nevertheless remains a menace to others so long as the bacilli remain in nose or throat. The practice of releasing an infected contact as soon as he is immunized is illogical, unjustifiable, absurd and dangerous. Similar tests of nose and throat (by smears instead of culture) may be made in the case of Cerebro-spinal Meningitis, the handling of the contacts and the conduct of release or isolation being similarly carried out on the basis of the results. Immune contacts (unless determined to be infective as above) may be released at once, after disinfection of their hands; and should be warned against the dangers of acquiring and carrying the infection on their hands (in poliomyelitis, cerebro-spinal meningitis, influenza, and diphtheria, in their throats and noses also) as a result of further contact with the case, or with carriers.

Non-immune contacts should be questioned carefully as to their dates of contact with the case; and the dates of infectiveness of the patient should be compared with these, in order to determine when the exposure began and when it ceased. If minute enquiry of this kind cannot be made, or is unsatisfactory for any reason, it is proper to assume, until proved otherwise, that members of the same household, office, etc., were all exposed on the first day the case became infectious, and continued to be exposed daily up to the date of isolation of the case.

Whether exact individual determinations of these dates of exposure be made, or the blanket assumption indicated be applied, the further calculations are as follows: To the date of first exposure (usually the date of onset in the patient) add the minimum incubation period of the disease in question: this will indicate the earliest date at which any infected contact can develop the disease and therefore the beginning of quarantine (or observation) necessary for such contact or contacts. To the date of last exposure (usually the date of isolation of the patient) add the maximum incubation period of the disease *plus* the maximum prodromal period, thus fixing the last day on which any contact will develop the typical symptom of disease and hence the end of the necessary quarantine (or observation) period. Theoretically the maximum incubation period would be sufficient but the prodromal period is included in practice because, in many diseases, the onset may be trivial and may therefore be overlooked, the contact being discharged just as he begins to be infective; if the full prodromal period be included, the most careless can hardly fail to observe that the disease was begun and that isolation must be carried out.

In those diseases where the incubation period is long, and is not infectious (for example, in mumps), there will often be found to exist a period after isolation of the original patient and before the first contact can become sick, of a week or even ten days or so. During this period non-immune contacts may be given *interim* permits, allowing them to go on with their usual lives up to the date when the first case may be expected to develop. On that date they should be quarantined (or placed under observation) for the necessary period.

In measles, and German measles similar methods may be followed. In Diphtheria and Scarlet Fever it is rare that the time so saved to the contact is long enough to be worth the extra risk and work involved.

Observation Versus Quarantine

In dealing with intelligent conscientious people, or with people under full control as in schools, armies, etc., non-immune contacts, if observed twice daily by competent medical men or nurses, throughout the period during which they may develop the disease, may be freed of all other quarantine restrictions. The family physician will generally coöperate with the Health Officer in this twice-daily inspection, thus saving much otherwise necessary restriction, to the great satisfaction of all concerned.

The quarantine periods and examples of calculations are appended. See Table.

Date of	(1) Isolation	(2) Typical symptom	(3) Earliest symptom	(4) Infection	(5) Beginning inspection of contacts	(6) Ending inspection of contacts	(7) Release of patient	(8) Incubation period		(9) Prodromal period	
Fixed by	Discovery	Inquiry	Inquiry or Col. 2 − Col. 9	Col. 3 − Col. 8	Col. 3 + Col. 8*a*	Col. 1 + 8*b* + 9*b*	Section 18	(*a*) minimum	(*b*) maximum	(*a*) minimum	(*b*) maximum
German measles.......	Say June 5	Say June 3	June 2 or 3	May 18 to 21	June 15	June 22	June 9 or 10	14	16	0	1
Mumps.......	Say June 5	Say June 3	June 2 or 3	May 9 to 21	June 15	July 1	± June 23	14	25	0	1
Chickenpox...	Say June 5	Say June 3	June 2 or 3	May 17 to 20	June 16	June 23	± June 17	15	17	0	1
Scarlet fever....	Say June 5	Say June 3	June 1 or 2	May 26 to 31	at once	June 14	± July 5 to July 19	2	7	1	2
††Smallpox.... (severe)	Say June 5	Say June 3	May 31 or June 1	May 18 to 21	June 11	June 22	during July	12	14	2	3
Real measles..	Say June 5	Say June 3	May 30 or 31	May 20 to 23	June 7	June 20	June 14	9	11	3	4
Whooping cough........	Say June 5	Say June 3	May 28	May 15 to 22	at once	June 26	± July 8	7	14	7	7
Diphtheria....	Say June 5	Say June 3	June 2 or 3	May 31 or June 2	at once	June 13	3 neg. cults.	1	3?	0	1?
Typhoid......	Say June 5	Say June 3	May 28	± May 15	at once	June 26	± July 22	5*	23*	7	7
Para-typhoid...	Say June 5	Say June 3	May 30 or 31?	± May 18	at once	± June 22	± June 26	± 14 days		3?	4?
Cerebro-spinal.	Say June 5	Say June 3	May 28 to June 3?	?	at once	± June 25	3 neg. smears	unknown†		0?	7?
Poliomyelitis..	Say June 5	Say June 3	May 28 to June 3?	± May 15	at once	± June 25	± July 8	unknown†		0	7

* Average 14.

† 2 weeks?

†† In mild smallpox, incubation is often 17–18 days; prodromes may last 6 days.

Regulations Regarding Visitors

Persons of all ages and both sexes, who are not immune to the disease in question, must be rigidly excluded from all houses where cases or non-immune contacts are isolated or quarantined.

Immunes are rigidly excluded from contact with the patient—indeed no one should see the patient except physician, nurse or other attendant, although the law admits the clergy, and of course undertakers, in case of death.

Where important affairs make it essential that a visitor other than those permitted by law, should interview the case, or otherwise come in contact with him, immunes should be selected to make the visit, and they should be required to obtain written permits from the M. O. H. and be guided in their conduct during and after the interview by the general rules such as are followed by doctors and nurses to avoid carrying infection to others.

In Case of Death

The body should be handled only by a skilled undertaker: the orifices should be antiseptically plugged and the body wrapped in a sheet wrung out of bichloride solution, 1 in 500. Metallic coffins, embalming, etc., are entirely unnecessary, merely adding to the expense of the stricken family, without affording any additional protection to the public health. If such unnecessarily refined precautions are insisted upon by the public health authorities, then the community, not the individual, should bear the expense.

The funeral should be private, only members of the household already in the house or proved immunes from outside being permitted to attend any ceremonies in the house.

After the house ceremonies are over, non-infectious members of the family, immune or not, may attend the funeral procession; and whoever likes to do so may join the procession outside the house and go with it wherever they please.

Local laws and ordinances often call for excessive precautions and of course must be obeyed. The above, however, include all the real essentials for health protection.

DISINFECTION

Two systems of disinfection have been long recognized, *concurrent* and *terminal*. The former concerns the daily, hourly attention to, and disinfection of, everything coming in contact with the patient, especially with his discharges and all that they may contaminate. The latter concerns the final disinfection of the patient's room, perhaps of the whole house, occupied by him during the attack, after the recovery of the patient.

Very much undue emphasis has been given to terminal disinfection. Large expenditures are made for this purpose and great faith placed in it, unfortunately to the exclusion of attention to, and reliance on, the infinitely more useful and logical concurrent disinfection, which, properly done, ought almost wholly to displace it.

Terminal disinfection should be done following tuberculosis of the lungs, anthrax and plague; in tuberculosis because of the great numbers and wide distribution of the bacteria thrown out by the patient, especially the careless patient; in anthrax because of the existence of resistant spores possibly attached to furniture, etc.; in plague because of the intense virulence of the organism and its tendency, like anthrax, to infect directly through the skin. In the ordinary diseases of the temperate zone, however, terminal disinfection cannot for a moment take the place of concurrent disinfection and is unnecessary if the former be properly carried out.

METHODS OF DISINFECTION

CONCURRENT DISINFECTION.—The disinfection of infected discharges, and of everything coming into contact with the discharges, whether the discharges be of the nose, mouth, bladder, or bowel, and whether the things which come into contact with the discharges be utensils, clothing, hands, furniture, etc., should be done at once, as soon as the discharges appear, or the articles, hands, etc., become contaminated.

Bladder and bowel discharges deposited directly in proper sewer-connected toilet-bowls require no disinfectant treatment; but the seat, door-knobs, toilet paper rack, flush pull and so on, which the discharges may reach, directly or through the patient's hands, should receive disinfection every time the toilet is used by such a patient. Where bed-pans or urinals are used and then emptied into such a toilet-bowl, disinfection should be done of the hands of the attendant who empties the pan, of the whole pan itself, and of any part of seat or bowl (not reached by the flush) contaminated by splash or dribbles from the bed-pan or urinal

Where outdoor toilets or indoor toilets not connected with a sewer are in use the discharges must always be disinfected—preferably by half-filling the bed-pan or urinal, before use, with a saturated solution of milk of lime (unslaked lime, in water, to saturation—cool and pour off the liquid parts) into which the discharges are received. Where such toilets are used by the patient directly, an *abundant* layer of powdered unslaked lime should cover the discharges as soon as they are deposited. Such layer should be an inch deep. Precautions regarding the seats, door-knobs, hands, etc., should be followed as above described. The difficulty in enforcing these precautions makes fly-screening a better plan.

Soiled bed clothing or other clothing, handkerchiefs, etc., may be rolled up and placed directly in boiling water; but if some interval must elapse before they can be boiled, they should be put directly into 5 per cent carbolic acid solution, or 0.1 of 1 per cent bichloride of mercury solution or other disinfectant of similar killing power for at least half an hour. Thereafter they may be handled as uninfected clothing.

Eating utensils after use should go directly into boiling water for several minutes and then be washed in the ordinary way. Spoons used for medicine, toys, thermometers, etc., which it may be inconvenient or impossible to put into boiling water, should be immersed in 5 per cent carbolic acid or 0.1 per cent bichloride solution for half an hour, then washed.

These solutions may be used also for the hands and a large bowl of one or both of them (carefully labelled, and out of reach of children, etc.) should be constantly ready; into this the patient's and attendant's hands should be dipped after every contamination.

Discharges from the nose and mouth should be collected on paper or rags and burned at once. If inconvenient to burn them, they should be dropped into carbolic or bichloride solutions as above, and disposed of as harmless after a half-hour's soaking.

It is difficult to specify every form of contact to be guarded against by disinfection, but the foregoing are the chief ones to watch for, and the principles given should be widely and intelligently applied—remembering always that the *discharges* contain the *danger*.

TERMINAL DISINFECTION.—Sulphur disinfection (4 pounds burned for every 1,000 cubic feet of space, in the presence of steam sufficient to saturate the atmosphere) is effective for disease bacteria—also for roaches, bedbugs, etc., and for mice, rats, etc. But it injures fabrics by bleaching them, and metals by tarnishing them. Formaldehyde vapor is now used in its place for disinfection; but flies, bedbugs, etc., are not successfully exterminated thus. The most recent approved method for use in the disinfection of houses is the Minnesota State Board of Health potassium permanganate formaldehyde method.

For each 1,000 cubic feet of space the following should be used:

Potassium permanganate (crystals)	11 ounces
Solution formaldehyde (U. S. P. 1900)	11 ounces
Water	9 ounces

Directions for use:

Prepare the room to be disinfected by sealing all cracks, windows, ventilators, etc., and all the doors but the one for exit, with wet newspaper strips; open all blankets, drawers, etc.; separate and open up all books, clothing, etc., in the room. Have wet strips of paper in readiness to seal the last door when the disinfection has been started and the operator has left the room. The windows should be left unlatched so that they may be opened from the outside after the disinfection is completed.

Use a metal pail with lapped (not soldered) seams, or an earthenware receptacle, holding not less than fourteen (14) quarts, in which to mix the above ingredients. Place the receptacle on bricks standing in a pan of water, but the receptacle should not touch the water.

Place the 11 ounces of potassium permanganate in the receptacle, distributing it evenly over the bottom.

Mix the formaldehyde (11 ounces) and the water (9 ounces), and pour this mixture over the potassium permanganate in the receptacle.

This done, the operator should leave the room as quickly as possible, sealing the door behind him with the wet strips of paper prepared in advance for this purpose.

The directions above apply to the disinfection of a room containing 1,000 cubic feet or less. If a room contains more than 1,000 cubic feet of space, use one of the above disinfecting outfits for each 1,000 cubic feet or fraction thereof. Do not attempt to use a double charge in a container of even double capacity.

In disinfecting a whole house, begin with the most distant room and having mixed the potassium permanganate, formaldehyde, and water in the proper receptacle, close the door of the room and seal it at once as directed above. Proceed in this way in the disinfection of all the rooms. Leave the seals unbroken on the window and doors for six hours, after which the rooms should be opened up and thoroughly aired. The temperature of the room at the time of disinfection should not be below 70°F.

No paper, cotton, cloth, wood, or other combustible material should be in or near the disinfecting outfit for fear of fire, and no flame should be permitted in the room near the disinfecting outfit.

Carriage of Infection by Biological Agents

The transmission of yellow fever and malaria by mosquitoes, in the course of which the parasite causing the disease must undergo a whole series of developmental changes before the mosquito can become infective, is now well understood. But the mechanical carriage of infectious material by flies from privy vaults or bed pans or even mucous membranes or open wounds to food and drink or to other mucous membranes or wounds has not been very long established.

That typhoid fever and dysentery have many times occurred in epidemic form chiefly by the carriage of the infective agents by flies the writer firmly believes as the result of personal investigation, as well as from the reports of others. Similar mechanical carriage of infection on the outside of the body has been attributed to rats, dogs, cats, even to cows and horses. This must not be confused with the dissemination of certain diseases by horses actually sick with the disease (glanders) or carrying the germs in their intestines (tetanus), by cows actually sick of tuberculosis, or by other similar instances of disease derived directly from preceding cases or carriers in the lower animals.

Another class of cases where lower animals convey disease by biting, and yet act merely mechanically is instanced by the septicæmia sometimes arising from bites of well animals (rats, snakes, mosquitoes, etc.), the bite acting merely to admit to the tissues pathogenic forms accidentally present in the animal's mouth or on the skin of the bitten person. These must be distinguished from cases where the animal transmits thus a disease from which it is itself suffering (as when a rabid dog spreads rabies by biting other animals or man) and from true poisoning by injection of animal products at the time of biting (as done by poisonous snakes, mosquitoes, etc.).

CHAPTER VI

MICROBIAL DISEASES OF MAN AND DOMESTIC ANIMALS

DISEASES CAUSED BY MOLDS* AND YEASTS

The diseases produced by fungi in higher animals are mostly localized infections of the skin (dermatomycoses), of the mouth and throat (thrush), of the lungs and air passages (pneumomycoses), and of the lymphatics (Sporotrichosis and Saccharomycosis).

PNEUMOMYCOSIS†

ASPERGILLOSIS.—The fungus disease of the lungs and air cells of birds is quite uniformly attributed to *Aspergillus fumigatus* which is widely distributed in the soil and upon feed and grains. The agency of this species in causing disease is well established. It grows best at blood-heat. Inoculation experiments have produced the disease in animals. Isolated cases are recorded in which this organism is regarded as the cause of disease in horses or cattle and even man. Biologic forms of A. fumigatus are very widely distributed. Many of them are readily separated by cultural characters. Pathogenicity is not limited to one or a few strains since lesions have been described as due to a wide range of the varieties of this group. Other species of *Aspergillus*, *A. flavus*, *A. nidulans*, *A. niger*, have been listed among pathogenic forms from their presence at times in diseased tissue. Whether these species are ever a primary cause of disease is doubtful, but their presence and activity in such infected areas has been established.

Secondary Infections.—Spores of any species of fungus found in the locality may find lodgment in wounds, orifices open to the outside, such as the external ear or the air passages. Many of these spores will germinate in such situations. If favored by dirt, pus, mucus, or existing pathological condition the resulting growth in some species develops into a secondary infection; most species lack entirely the power to produce disease. The appearance of molds, especially species of

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* Arranged generically as far as possible.

† Prepared by Charles Thom.

Mucor, *Penicillium*, and *Aspergillus*, in such situations has been frequently reported in literature. In very large measure at least such presence may be regarded as evidence of lack of care, cleanliness, of even ordinary precautions when the infection involves man.

THRUSH*

The parasite of thrush, *Oidium albicans* Robin, (*Saccharomyces albicans*, Reiss), in culture produces a scanty mycelium, submerged in the substratum, which branches monopodially. The tendency to budding and to the entire suppression of the mycelium leads some to regard this form as a yeast (Fig. 161). It attacks the mucous membrane of the mouth and throat in young animals only, producing vesicles, then white membranous patches composed of the mycelium of the fungus (Fig. 162). It is to be recognized in such cases by microscopical examination. The same disease affects children and is found in fowls, calves, and colts.

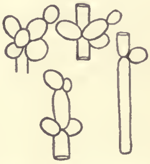

FIG. 161.—*Oidium albicans*, from a culture obtained from Kral.

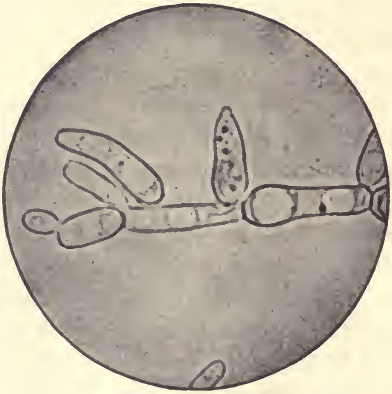

FIG. 162.—*Oidium albicans*. (*Kohle and Wassermann.*)

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* Prepared by Charles Thom.

Although this disease has been more common in the tropics, enough cases are known from the American mainland to indicate an increasing importance.

Dermatomycoses*

The molds which cause skin diseases form a small group, with very ill-defined relationships to the commoner forms of fungi. They produce a vegetative mycelium within the tissues of the host with fertile branches which bear conidia but indicate little as to their group relationships among fungi. Certain of these diseases have been carefully studied, mostly from the pathological side, but a very large number of such lesions are recorded without adequate study of the organs involved. Most of these diseases are tropical but considerable numbers of cases have been recognized in temperate America in recent years. A few of these diseases are practically cosmopolitan.

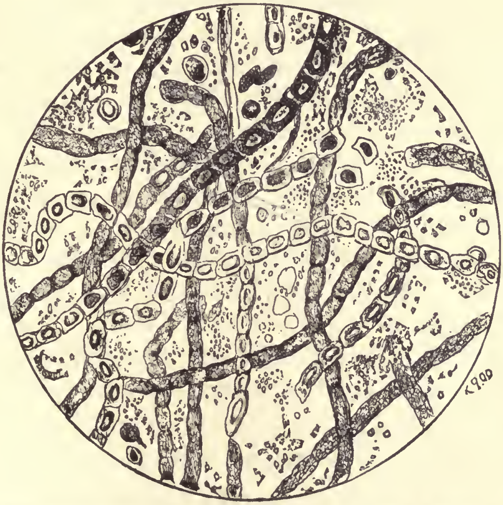

Fig. 163.—*Trichophyton tonsurans.* (*After Hyde, from Adami and Nicholls.*)

Barber's Itch, Ringworm, Herpes Tonsurans, Trichomycosis. The disease due to *Trichophyton tonsurans* (Fig. 163), Malm, has received many names in different languages. It attacks man and domestic animals, the ox, horse, dog, cat, sheep, hog, probably other animals as well. It is characterized by the formation of circular patches from which eventually the hairs fall. These patches enlarge radially and fuse into large areas covered with crusts with more or less discharge in the center. The fungus is recognized microscopically by examination of hairs pulled from the growing edge of the infection. The hyphæ penetrate the layers of the skin and especially surround the roots of the hairs which, when first affected, stand stiff and straight.

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* Prepared by Charles Thom.

The appearances of the disease differ in the various species of infected animals, as also does the length of time it continues. The disease does not affect the general health greatly, since it primarily attacks the drier and more horn-like portions of the skin, but becomes conspicuous by the falling of the hair and by the scabs or crusts with accompanying itching and discomfort. Other species of the same genus have been described which produce infected areas differing in detail but similar in their general characters.

.FAVUS.—Favus is caused by *Achorion schönleinii*, Remak, and affects man, cats, dogs, mice, rabbits, and fowls, and many wild animals. This is characterized by crusts, thickened at the edges and somewhat cup-shaped in center, composed of the mycelium of the mold cemented together into masses by glairy substance. Below, these crusts are in contact with the true skin. The fungus penetrates especially into the hair-follicles and hairs themselves, which later are shed. It attacks different species of animals with varying symptoms, but produces more serious lesions than those of *Trichophyton*. Favus is especially serious as it attacks man. Efforts to show that this fungus is merely a parasitic form of some species of higher fungi have failed. The diseased conditions have become so well defined and are reproduced so uniformly as to indicate a fixed habit in the organisms, whatever its source or relationship.

ACTINOMYCOSIS*

Actinomyces bovis†

This is a rather common disease of domestic animals, especially cattle. It prevails in Europe, North and South America, and is known by various names as lumpy jaw and wooden tongue. Cattle are most commonly affected, but humans, hogs, horses, sheep, and dogs are susceptible. *Actinomyces* produces a local disease which never spreads widely or rapidly.

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* Prepared by M. H Reynolds.

† *Actinomyces bovis* has been classified by Frost (page 111) as a species of bacteria, but, because of many features, it is here inserted with the organisms strictly belonging to molds and yeasts.—*Ed.*

Actinomycosis is to be considered as an infectious disease which spreads by inoculation.

The disease produced by this microörganism usually runs a chronic course and is distinguished especially by enlargement of affected parts, by hardening of the tongue, and by suppuration. The latter is one of the most constant and conspicuous characteristics. Head parts, including the facial bones, are commonly affected; lungs and various other internal organs and even the vertebræ may be involved.

The extent of injury done by this fungus depends on the location and size of the involved area. Usually the most conspicuous injury is impaired nutrition.

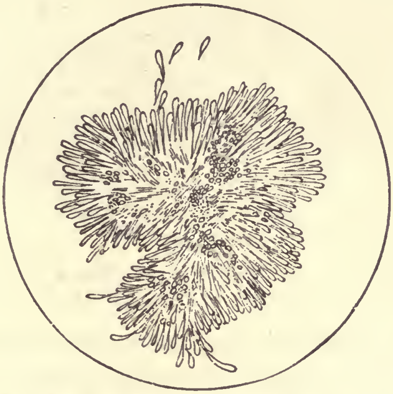

FIG. 164.—*Actinomyces bovis.* The ray-fungus from cow. (Diagrammatic.) (*After Williams.*)

There is probably but little risk to human health from actinomycosis in cattle as parts of the carcass most commonly affected are not eaten and edible parts are usually cooked. It is generally considered that sound portions of carcasses which do not show generalized disease are fit for human food purposes.

There are apparently several varieties of *Actinomyces* all of which are recognized for the present as *Actinomyces bovis.*

The varieties of *Actinomyces* are to be regarded as members of a very complicated group of microörganisms higher than bacteria and are generally spoken of as fungi. *Actinomyces bovis* is commonly known as the ray-fungus (Fig. 164). Its relation to the disease of actinomycosis is probably specific but it is frequently aided by pus producing bacteria.

It is believed that the *Actinomyces* vegetate on various grasses, especially wild barley, and that infection occurs by inoculation with the awns and barbs of such grasses through the mucous membrane of the mouth or other portions of the alimentary tract.

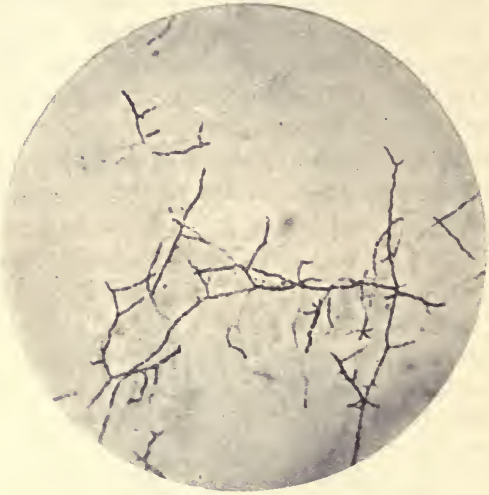

FIG. 165.—Actinomycosis. *Actinomyces bovis.* Preparation from a pure culture. ×1000. (*After Williams.*)

Infection by inoculation is the most common method of introducing the disease; but infection by inhalation evidently occurs in some cases. It seems probable that some special stage of development for the *Actinomyces* is necessary either within the diseased animal body or upon some plants, in order that it may be able to infect animal bodies, for direct inoculation by pus has usually given negative results. Inoculation by bits of diseased tissue occasionally gives positive results.

It is evidently not a producer of active toxins for the disease disturbances are apparently due to harmful growth in the tissues and to secondary infection.

Suppuration is one of the conspicuous features as is also the development of much new granulation tissue which tends to degenerate at the center. Soft organs affected by this parasite show a tendency to multiple abscesses.

Actinomyces bovis grows rapidly on a variety of laboratory media. On glycerin agar the colonies develop into transparent drop-like bodies in four or five days at 37°. Old colonies become white or yellowish with a powdery surface. The cultural and other peculiarities vary much and according to the variety under observation. Some varieties appear distinctly aerobic and others anaerobic. As a rule it liquefies gelatin growing in spherical masses which settle to the bottom of the liquid. Filaments appear in artificial growth which are very long and slender, and about 5μ in diameter, and show true branching (Fig. 165). The young colony is a loose mass of filaments; older colonies become dense and felted. Rod-shaped and spherical forms appear in artificial cultures. Cultures, especially those containing the round forms, are very resistant to heat, light, drying and disinfectants. Stains easily. Tissue section stained with carmine followed by Gram's method gives good results, the thread showing dark and clubs red. Carmine followed by Weigert gives a beautiful stain. May be recognized as visible granules found floating in the pus in case of suppuration, or embedded in tissue. These granules vary in color; some are clear or yellow; others are quite dark. The colony as it appears in tissue section or pus smear consists of a rosette arrangement. The central portion of the colony is a dense mass of mycelium and spherical bodies. From this felted central mass, there extend rays or club-like bodies. Club-shaped enlargements at the ends of filaments frequently appear and are regarded as a distinguishing characteristic of *Actinomyces*. This organism is usually destroyed at 75° for thirty minutes. Final diagnosis must rest upon actual demonstration under the microscope which is not difficult. The granular masses may be washed in normal salt solution; and examined unstained, or stained in diluted carbol fuchsin.

Escape from the diseased body is usually in pus discharged from actinomycotic abscesses. In case of open lung or intestinal lesions it may be discharged through the trachea or intestines.

Actinomycotic pus scattered over fodder, mangers, and feed racks probably serves indirectly as a source of dissemination.

Actinomycosis is not a disease of rapid or extensive dissemination. Control work is usually confined to isolation, to proper disposition of diseased animals and to suitable disinfection.

ACTINOBACILLOSIS.—Actinobacillosis is probably to be distinguished from actinomycosis. It is very similar in subjects affected, in history and clinical evidence, but apparently different as to specific cause. The cause of actinobacillosis seems to be a very small bacterium found also in rosette-like masses resembling those of *Actinomyces*.

MYCETOMA (MADURA FOOT)*

This disease is endemic in India, especially in Madura, and is found in other warm countries.

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* Prepared by Edward Fidlar.

It is a chronic inflammatory process found most commonly in the foot, occasionally in the hand but very rarely elsewhere. It is characterized by swelling and irregular deformities of the part with the occurrence of sinuses whence there is a purulent discharge containing granules suggesting those of actinomycosis. These granules may be whitish, yellowish, reddish, or black in color.

The causative organism is generally regarded as a fungus. It is not unlikely that some cases of the disease may be confused with actinomycosis. Several different molds have been described, some of which have been classed as *Aspergilli*, while others have been given new names. It is probable that, while the disease is a fairly well-marked clinical entity, the etiological agent varies in different localities.

Successful inoculation of the monkey with the white variety and of pigeons with the black variety has been recorded.

MYCOTIC LYMPHANGITIS*

Saccharomyces farciminosus†

The disease caused by this yeast-like fungus has been called Japanese farcy, epizoötic lymphangitis, and mycotic lymphangitis. This disease was first recognized in the United States in 1907. It has already been found in Pennsylvania, Iowa, California, and North Dakota.

Saccharomyces farciminosus produces a slow, chronic, contagious disease of horses and mules. Cattle appear susceptible but rarely show clinical symptoms of infection.

This *Saccharomyces* involves especially the superficial lymphatic vessels and glands, but internal organs are occasionally affected. The disease is essentially local, constitutional disturbances being slight. The disease produced is fatal in about 10 to 15 per cent of cases affected but is much more serious than these figures would indicate. Other horses that do not die are rendered useless for service, the sale value being ruined in many cases.

The lesions produced by this parasite resemble most closely the farcy form of glanders but may be easily distinguished by quite different ulcers. The pus is thick, creamy, and usually yellow, whereas the pus from the farcy buds is clear and viscid. Farcy cases respond to the mallein test; lymphangitis cases do not.

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* Prepared by M. H. Reynolds.

† Work done by Paige, Frothingham and Paige, Meyer and others raises questions concerning specific etiology and proper classification, but it is deemed wise to continue this recognition and classification for the present. Various authorities classify this organism as cryptococcus, blastomyces, etc.

It seems to have been well established that *Saccharomyces farciminosus* is the direct cause of mycotic lymphangitis—at least of one form of it.

The *Saccharomyces* grows in the animal tissues and by its presence and products acts as a direct exciting cause of the disease. Entrance is effected through wounds which may be very superficial and very trivial, most frequently perhaps on the legs, shoulders, and neck. The incubation period varies from a few weeks to several months.

This *Saccharomyces* is distributed through lymph vessels, chiefly superficial ones, the nodules appearing first near the point of inoculation.

The tissue changes produced are infection, inflammation, and suppuration of the lymph vessels and glands. At first the lymph vessels enlarge and harden; then nodules develop under the skin along the course of the vessels. These nodules suppurate and the small abscess cavity fills up with bright red granulation tissue. An entire limb may enlarge very greatly by reason of excessive connective-tissue formation, and the greatly thickened skin.

Saccharomyces farciminosus is a yeast-like fungus, ovoid in shape and 3μ to 5μ long by 2.5μ to 3.5μ broad. This fungus grows slowly under artificial conditions on agar and bouillon after inoculation with pus from an abscess. It reproduces by budding and does not stain well by common laboratory stains. Claudius' method of staining gives good results.

Cases should be isolated and stables disinfected by the free use of very strong disinfectants as this *Saccharomyces* is not easily killed by ordinary disinfecting solutions.

Another mycotic organism has more recently been reported* in the United States as causing a lymphangitis very similar clinically to the lymphangitis caused by *Saccharomyces farciminosus*. Cases supposed to have been plain cases of the *Saccharomyces* form showed on laboratory examination a *Sporothrix* acting as the direct cause. These workers* reproduced cases by inoculation and recovered an organism differing very materially from *Saccharomyces farciminosus*.

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* Sporothrix and Epizootic Lymphangitis, Paige, Frothingham, and Paige. Journal of Medical Research, Vol. XXIII, No. 1. This has been previously reported by Shenck, Hektoen, and others for the human.

The case history and lesions produced parallel very closely those produced by the *Saccharomyces*. This *Sporothrix* seems to have great vitality, remaining virulent in dried pus at a temperature of −7° for three months or more.

The same organism has been recovered from similar lesions of the human where it was apparently acting as the direct exciting cause. If this be confirmed, we have two apparently different organisms capable of producing a similar mycotic lymphangitis. Complement fixation work by Meyer suggests that the two may be at least more closely related than was previously supposed.

DISEASES CAUSED BY BACTERIA*

BOTRYOMYCOSIS†

Micrococcus pyogenes

We have typically in this disease closed abscesses with very tough fibrous walls and slow development. These abscesses involve especially subcutaneous and intermuscular connective tissue, although typical lesions have been found in various internal organs.

This affection is probably limited to equines. The essential characteristic of this disease is the presence of the peculiar masses of micrococci (Bollinger's granules). This massing seems to occur only in chronic cases where a certain degree of immunity has developed.

The identity and proper classification of a specific microörganism is still in dispute. Johné found *M. ascoformans* acting as an etiological factor. Kitt and others found micrococci which could not be distinguished from *M. pyogenes*. Moore found a variety of pyogenic micrococci and streptococci apparently serving as causative agents and reports one case of an enlarged spermatic cord where he found a fungus resembling *Actinomyces bovis*. Others identify *Botryomyces equi* as *Staphylococcus pyogenes aureus*, etc.

Primary infection occurs by inoculation and not infrequently follows surgical operations, *e.g.*, castration. The primary infection may then lead to involvement of internal organs by metastasis. The local effect here is that of an irritant and both irritant and tissue response appear to resemble those that occur in actinomycosis.

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* Arranged alphabetically under each of the following families: *Coccaceæ* (*Micrococcus*, *Streptococcus*), *Bacteriaceæ* (*Bacterium*, *Bacillus*, *Pseudomonas*), *Spirillaceæ* (*Microspira*).

† Prepared by M. H. Reynolds.

Botryomycosis is easily distinguished from actinomycosis on microscopic examination. Cases that resemble the farcy form of glanders are easily distinguished by mallein test, by laboratory animal inoculation and by lack of adjacent lymph-gland involvement.

GONORRHŒA*

Micrococcus gonorrhœæ

Gonorrhœa is one of the most prevalent of the bacterial diseases and is found throughout the civilized world and is confined to the human race.

The urogenital tract is the most frequent seat of infection but orchitis, conjunctivitis, and arthritis are not uncommon and endocarditis and a septicæmic condition may also occur. Ophthalmia neonatorum is due to this organism. The ordinary infections of the urogenital tract have an incubation period of from two to eight days. The inflamed mucous membranes give rise to more or less pain and yield a thick yellow discharge.

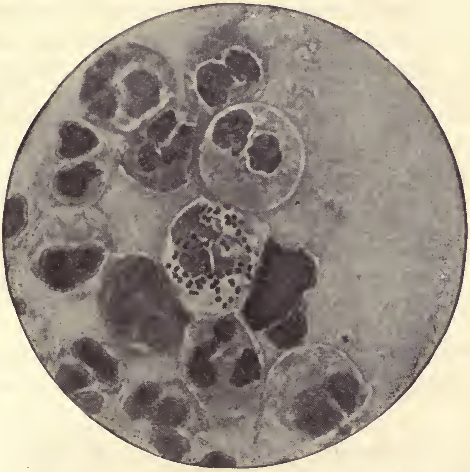

FIG. 166.—*Gonococci* and pus cells. × 1000. (*After Williams.*)

While the fatality due directly to *Gonococcus* infection is not high, the frequent tendency to chronicity and its connection with blindness and sterility render it one of the most important diseases.

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* Prepared by Edward Fidlar.

Gonorrhœa has been known from the very earliest times. In 1879 the diplococcus was pointed out by Neisser as the probable cause. Bumm in 1885 first cultivated it on coagulated human placental serum.

The microörganisms can be easily stained in the typical early discharges where it occurs in pairs and for the most part within cells (Fig. 166).

For isolation, sterile human blood, blood-serum or ascitic fluid should be added to melted nutrient agar at about 45°. Thompson's plasma glucose agar and Wassermann's swine-serum-nutrose medium are also good. The *Gonococcus* is about 0.6μ to 0.8μ in diameter. It is usually seen in pairs; where the adjacent sides of the cocci are flattened the long diameter of the pair reaches as much as 1.6μ; non-motile, and forms neither spores nor capsules. It stains readily with the aniline dyes and is Gram-negative. The temperature range is 30° to 38.5° with an optimum of 37.5°. Aerobic conditions are preferred with a reaction about 0.6 per cent. acid to phenolphthalein, but Ruediger reports luxuriant growth in air-tight-tubes of special neutral serum agar. On serum agar or similar suitable media, colonies appear in twenty-four hours as fine slightly elevated, translucent or opalescent spots frequently referred to as "dew-drop" colonies. They possess a faint bluish or grayish white color with a slightly marked concentric or radial striation with a scalloped margin and finely granular center. In serum broth there may occasionally be a uniform clouding though, as a rule, there is a finely granular, somewhat slimy sediment with clear fluid above. Only in exceptional cases has growth been observed in gelatin because of the unfavorable temperature. On inspissated blood serum growth may sometimes be observed as discrete pale yellowish or brownish colonies. Dextrose is changed with the development of acid but no gas. Alkali is not formed in any medium by typical strains. No gas, indol or pigment are formed. The toxins are intracellular and quite thermostable. Resistance is very slight toward external influences. Cultures undergo rapid autolytic changes and die out at room temperature, often within forty-eight hours. A temperature of 41° to 45° will kill in a few hours. To light and drying they are also very sensitive, and are rapidly killed by the ordinary disinfectants.

Animals inoculated subcutaneously or intraperitoneally show symptoms of poisoning with suppuration and necrosis locally and may succumb.

The virulence of the organism is variable. They may apparently lie dormant or rather, may remain very slightly active in chronic conditions in one individual but set up an acute gonorrhœa when transferred to a second person.

The organism gains entrance to the urethral mucosa or conjunctiva usually by direct contact and it is doubtful if the disease could be carried by any infected article later than twenty-four hours.

The organism is found at the local lesion and has been obtained from the fluid of affected joints, and from the blood in the septicæmic cases.

A general immunity is seldom if ever developed in man following an attack of gonorrhœa. A complement-fixing antibody has been demonstrated, however, which is sometimes an aid in diagnosis when a good polyvalent antigen is used in the test. Thomson's antigen is an improvement.

Injections of cultures into animals give rise to agglutinins, bactericidal and complement-binding bodies.

The diplococcus is eliminated only in the purulent discharge.

Great importance attaches to the fact that persons may harbor the *Gonococcus* long after the acute condition has disappeared and when the coccus seems to be no longer harmful to its host. Such cases bring about untold misery and form one of the most difficult problems in both medical and social science. It has been stated that *Gonococci* have been found as late as twenty years after the primary infection.

The most extended and successful prophylactic measures have been carried out in the armies and navies of various countries by the use of germicidal solutions whenever there has been any chance of exposure to infection. The use of germicidal solutions in the eyes of newborn infants is practically universal as a preventive measure against ophthalmia.

EPIDEMIC CEREBRO-SPINAL MENINGITIS*

Micrococcus meningitidis

Cerebro-spinal meningitis may be caused by different bacteria such as the pneumococcus, streptococcus, staphylococcus, influenza bacillus, tubercle bacillus, etc., but the greater proportion of cases of acute meningitis, those of the epidemic type, are due to the meningococcus or diplococcus intracellularis meningitidis.

Epidemic meningitis has been described chiefly in Europe and America and appears to have been first clearly defined in 1805. While sporadic cases occur, the disease usually exists in the epidemic form, beginning in the fall, continuing during the winter, and declining in the spring. Of late years it would seem to be on the increase.

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* Prepared by Edward Fidlar.

The incubation period is unknown.

There is considerable variety in the character of the cases. As a rule the invasion is sudden with headache and vomiting as prominent symptoms. The headache usually increases, with disturbances of vision, restlessness, and pains and rigidity in the muscles of the back and neck. The temperature is irregular and variable, the usual being about 101° to 102°. Herpes occurs frequently and a purpuric rash is common, especially in the severe cases, so that the term "spotted fever" has sometimes been given to the disease. The patient usually passes into a stuporous state, though delirium may occur before it. Death may occur in a few hours (fulminant type) or within a week, or occasionally may be postponed as late as six months. In all favorable cases the recovery is slow.

A fibrinous exudate which occurs chiefly at the base of the brain, and the presence of pus cells in the cerebro-spinal fluid, are prominent pathological features, but in the fulminant cases the gross pathological findings may be surprisingly insignificant.

According to Flexner, serum treatment has reduced the mortality of the disease from about 70 per cent to 30 per cent, and to less than 20 per cent if treatment is begun within the first three days.

The demonstration of the organism in the cerebro-spinal fluid of the typical case may sometimes be an easy matter, but at other times may require a prolonged search. It appears as a Gram-negative coccus, single and in pairs, frequently within pus cells but occasionally extracellular. From the usual case it can be obtained in pure culture by sowing the sediment from the spinal fluid upon suitable media. The amount of material planted should be abundant and to supplement these first cultures it is well to incubate the fluid at 37° for twelve to eighteen hours and then make further inoculations. The media used are often blood agar or serum glucose agar, but legumin-trypagar was most successful in the British army during the war.

As found in culture media the meningococcus will show swollen involution forms often in comparatively young cultures. There are no spores, flagella nor capsules. It can be stained readily by the aniline dyes and with methylene blue will sometimes show metachromatism. It is Gram-negative.

The temperature relations are of some importance in identifying the coccus. It has a minimum temperature of about 25°, an optimum of 37° and a maximum of 42°. Its atmospheric requirements are those of an aerobe.

On serum agar the colonies are small, grayish and glistening, with smooth outline and granular center. On legumin-agar they are round, hemispherically raised, and opalescent. In broth growth is slow and occurs at the surface. Only rarely is growth obtained on gelatin media chiefly because of the unfavorable temperature required. There is no change in litmus milk. Acid is formed from dextrose and maltose.

The toxins of the meningococcus are probably intracellular.

The resistance of the organism to unfavorable conditions is very slight, and it undergoes autolytic changes almost with the same rapidity as does the gonococcus.

Meningitis due to this coccus does not occur naturally in animals, but it has been produced in monkeys artificially. Laboratory animals inoculated subcutaneously, intraperitoneally or intravenously with a sufficiently large dose will die without developing meningitis.

Animals immunized by graded doses show specific agglutinins, opsonins and lysins. Horses so treated yield a serum which in some hands has given very favorable results. In the first epidemics in the British armies however, the mortality ranged from about 40 to 50 per cent and the serum was distinctly disappointing. Investigation then demonstrated that four types of the meningococcus existed, distinguishable by agglutination reactions, and new sera were produced which yielded better results.

As the germs leave the body in the discharges of the nose and mouth, the prevention and control of the disease would appear at first thought to be an easy matter, but the occurrence of carriers and ignorance of the factors which govern the virulence of the infective agent and the individual's susceptibility make epidemic meningitis a very difficult problem from the standpoint of public health.

INFECTIOUS MASTITIS*

Infectious mastitis or mammitis (inflammation of the udder) appears in isolated outbreaks and is serious for the individual owner and individual herd, but it never spreads widely. It may affect a large portion of the herd and cause heavy financial losses. Infectious mastitis may have serious significance for children and others consuming milk; but there is little information on this point, based on careful work.

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* Prepared by M. H. Reynolds.

This is to be considered as an infectious, enzoötic disease and probably not specific. There is good reason to suppose that different outbreaks have been due to several different pyogenic or pus-producing organisms.

We cannot consider any one species of bacteria as the specific cause. Various micrococci, streptococci, and staphylococci have been found acting as causal agents.

Recent evidence indicates that udders of apparently healthy cows may contain a variety of bacteria and that the infections may remain more or less permanent. This is in part the explanation of recurrent cases of mastitis.

Discharge is either through the teat or rarely by external rupture of abscess. Transmission from cow to cow is indirect, and frequently on milkers' hands.

Entrance is usually effected by way of the milk ducts, thence into the milk cistern and to more remote parts of the gland. The infection may also come by way of the blood or lymph channels to the glands. A given case may thus be due to bacteria previously in the udder, the attack being determined by an area of lessened tissue resistance produced by injury.

In one class of cases, the gland structures are first involved; in other cases the connective tissue frame-work is first involved. In one type of this disease caused by streptococci these microörganisms attack especially the mucous membrane lining milk ducts and produce a catarrhal disease of that membrane. This is indicated by a cord-like swelling which extends along the milk canal through the teat to the milk cistern. This infection frequently leads to "blind quarter;" *i.e.*, to closure of the teat canal and loss of the quarter; or this infection may lead to the formation of one or more pea-like nodules along the teat canal and consequent obstruction.

In many cases the lactose is decomposed by the invading organisms, leading to the formation of organic acids. These acids produce coagulation. The coagula soon obstruct the milk ducts and alveoli and the secreting cells degenerate. The invaded tissues may suppurate or even become gangrenous.

These infections are indicated by dullness, lack of appetite, fever, inflammation of the udder, and by small nodules or cord-like swelling within and lengthwise of the teat.

It must be borne in mind that the infecting microörganism is the thing to be controlled. Outbreaks of this disease frequently have origin in infected cows added to the herd. Some cows are unsuspected "carriers." New cows should be suspected until found free by careful examination.

Affected cows should be isolated if possible, and always milked last. Their milk should be boiled and fed to hogs, and the milker's hands suitably disinfected.

MALTA FEVER*

Micrococcus melitensis

This disease is endemic along the shores of the Mediterranean, in South Africa, India, China, the Philippines, and the West Indies.

The period of incubation is usually about six to ten days.

The ordinary variety shows an intermittent or undulatory fever which may be protracted to six months or more, accompanied by constipation and general debility with various complications such as neuralgias, arthritis, orchitis, etc. Relapses occur after periods of absence of symptoms. Malignant cases are described which may be fatal in a week or ten days. The mortality is 2 per cent and no characteristic pathological changes are found.

The etiological factor is *M. melitensis* and was described by Bruce in 1887.

The organism can be obtained from the blood and in many cases from the urine. The most recently reported favorable medium for blood cultures is peptone broth with the addition of bile.

It is generally recognized as an oval coccus, although it is also described as a bacillus. Its maximum measurements have been found to be 0.8μ by 0.53μ, its minimum diameters 0.55μ by 0.4μ. It occurs singly, in pairs, in irregular groups and in short chains. (Recently the organism has been described as motile and possessing a single flagellum at the extremity of the long diameter of the oval coccus.) It stains by ordinary aniline dyes and is Gram-negative. It grows slowly at room temperature, better at body temperature and does not seem to be markedly sensitive to acidity or alkalinity of reaction. It grows aerobically. On plain agar after about forty-eight hours small whitish to yellowish colonies appear. Growth has been observed in broth in eighteen to twenty-four hours, on gelatin in eight or nine days, and the latter is not liquefied. It has been found to grow on acid potato and in acid or alkaline urine.

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* Prepared by Edward Fidlar.

Human beings and animals eliminate the organisms in the urine, and the milk of goats has been found to be a prolific source of infection. With proper regulations in regard to goats' milk the disease has been greatly reduced.

STAPHYLOCOCCIC INFECTIONS*

Boils, Abscesses, Wounds, Osteomyelitis, Pyemia, Etc.

Micrococcus pyogenes var. aureus, etc.

Infections of this order are found throughout the world and because of the association of staphylococci and streptococci with the large majority of purulent inflammations, these organisms are called the pyogenic cocci.

No specific disease is produced, but chiefly boils, circumscribed abscesses, infected wounds, osteomyelitis, pyæmia, etc. The symptoms alone will not indicate whether staphylococci or streptococci are present, but a low grade of infection with more pus and less constitutional disturbance tends to indicate the former, and staphylococci tend to pyæmia rather than to septicæmia.

Pasteur, Koch, Ogston and Rosenbach established the importance of these organisms.

Staphylococci in pus stain readily with aniline dyes. Pure cultures can be obtained by plating or streaking on plain nutrient agar.

While several different forms are found in pathological conditions, the *M. pyogenes var. aureus* is by far the most frequent, and it is described here as a type.

M. pyogenes var. aureus is a spherical coccus about 0.7μ to 0.9μ in diameter though forms 0.4μ to 1.2μ have been noted. On solid media the organism may be found solitary, in pairs, or in rows of three or four, but characteristically in irregular groups like bunches of grapes. In liquid media the single and paired arrangement is most frequent. No spores, no capsules and no flagella are found; the organism shows marked Brownian movement, like other cocci; Gram-positive. The temperature range of growth is from about 10° to 43° with an optimum about 30°. Aerobe and facultative anaerobe. It grows readily on all routine media, preferring a reaction slightly alkaline to litmus. Growth on plain agar is rapid and abundant. After twenty-four hours there appear round grayish-white or yellowish colonies about 2 mm. in diameter, smooth and raised above the surface of the medium. Microscopically, the colonies are regular in outline and finely granular. The characteristic orange-yellow pigment may not appear until later or if already present in twenty-four hours, deepens with further growth. In broth, growth is also rapid and causes a diffuse clouding with a thin pellicle and a heavy sediment after several days. In gelatin, colonies are as on agar and sink into cup-like depressions as the medium is liquefied. Liquefaction is rapid with some strains and slower with others, and in old cultures is of a funnel or saccate type. It is due to a thermolabile ferment-like substance known as gelatinase. In milk, the staphylococcus grows readily and causes coagulation sometimes early but usually in three or four days' time. On potato growth is usually abundant; it is not as moist nor as smooth as on agar and is slower. Pigment is developed usually to the highest degree and sometimes cultures appearing white on agar develop pigment on potato. On inspissated blood serum growth is usually moist and abundant. Occasionally the growth sinks slightly into the medium suggesting partial liquefaction. In dextrose, lactose and saccharose media, acid is produced, but no gas. Acid is a constant product. Formic, lactic, butyric and valerianic acids have been found and probably other fatty acids occur. Some authorities state that indol is formed but negative results are the rule. Nitrites are formed by the reduction of nitrates. A characteristic odor from cultures is due probably to the presence of fatty acids. The pigment appears in aerobic cultures and is absent in anaerobic cultures. It is insoluble in water but soluble in alcohol, chloroform, ether and benzol. The toxins are largely intracellular. A thermolabile, hæmolytic substance may be found in the more virulent strains after about ten days' growth in moderately alkaline broth and can be freed by filtration through porcelain filters. Another soluble toxic substance is found, causing the death of leucocytes—*leucocidin*. It is considerably less stable than the *staphylo-hæmolysin*. The staphylococci are among the most resistant of the non-spore bearing bacteria. Sometimes 60° for a full hour or even longer is necessary to kill watery suspensions; 70° is usually necessary to kill in ten minutes. If organic material is present the resistance is, of course, much greater. Low temperatures have little effect and it has been stated that 30 per cent have survived thirty minutes' exposure to liquid air. To direct sunlight and drying staphylococci also show considerable resistance and may be found in dried pus for several months. Resistance to germicides is also somewhat greater than that of other vegetative bacteria, and is increased especially in the presence of organic material. In watery suspensions staphylococci are killed by 1 : 1000 mercuric chloride in ten to fifteen minutes, by 3 per cent carbolic acid in two to ten minutes and by 5 per cent formaldehyde in the same time.

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* Prepared by Edward Fidlar.

Man seems to be considerably more susceptible to staphylococcic infections than animals. Of the latter rabbits and mice and guinea-pigs are susceptible in this order.

The virulence of the organism shows considerable variation and is usually increased by successive passages through animals of the same species while remaining unaltered for animals of other species.

Subcutaneous inoculation usually results in abscess formation. Virulent cultures injected into the peritoneal cavity of animals may kill in forty-eight hours to a week or even longer, with pyæmic abscesses especially in the kidneys. Malignant or ulcerative endocarditis has been experimentally produced by intravenous injection when the heart valves have been injured, chemically or mechanically. Osteomyelitis has also been experimentally produced.

In man simple rubbing of virulent cultures into the skin is often sufficient to produce a furuncle.

Upon entering the tissue the cocci are strongly chemotactic and pus inevitably results. With virulent cultures the leucocidal substance is more or less strongly active. The organism may be limited to the first abscess or by invasion of the blood stream multiple abscesses result. In these cases, which are usually fatal, the organism will be found throughout the body.

Immunization can be secured by repeated injections of cocci dead or alive in graduated doses. The sera possess slight bactericidal and agglutinating properties, and a high degree of opsonic power. The latter property is probably the most important.

The serum of immunized animals is protective only when used slightly before or along with the injection of the organisms and is consequently of little practical value. Active immunization, however, is being extensively practised particularly with the autogenous strains. Leucocytic extracts have also been successfully though not so widely used.

The prophylaxis of staphylococcic infections is the same as for other pus-producing forms.

Several other kinds of staphylococci have been found associated with pathological conditions, the most important of which are *M. pyogenes var. albus*, *M. epidermidis albus* (Welch), and *M. pyogenes var. citreus*. The first seems to be slightly pathogenic, and rarely produces severe infection. It is distinguished from the aureus by lack of pigment.

The second variety appears to be an attenuated form of the other.

The third variety is distinguished from aureus and albus by the development of a lemon-yellow pigment.

STREPTOCOCCIC INFECTIONS*

General Septicæmia, Puerperal Septicæmia, Erysipelas, Bronchopneumonia, Etc.

Streptococcus pyogenes

Several different methods have been used to classify streptococci. The species *Strept. viridans* and *Strept. hæmolyticus*, for example, are based on the action upon hæmoglobin. Fermentation of carbohydrates has claimed much attention, but no satisfactory correlation has been found between a biochemical classification and the clinical forms of infection encountered. Work in the British and American armies suggests that an immunological basis of division will prove better, and at present four types are indicated. Some French authors desire to separate a group called *Enterococcus* as distinct from, and of less pathological significance than *Streptococcus.*

Streptococcic infections are endemic among all races and under all social conditions. In the days before antisepsis and our knowledge of the transmission of infectious diseases, erysipelas and puerperal septicæmia occurred in epidemics that were the scourges of surgical and lying-in hospitals.

When the work of Pasteur and Lister became fully comprehended such epidemics ceased to exist.

Natural streptococcic infections have been described in horses and cattle and among the laboratory animals, but as a rule such disease is much rarer in animals than in the human being.

For septicæmia and erysipelas the period of incubation is probably from several hours to three days. For some conditions it is impossible to determine.

The symptoms of septicæmia begin with a rapid rise of temperature which may reach 105°F. or even higher. Chills accompany the fever and are often severe. The pulse is rapid, irregular and weak and the respiration labored. There may be vomiting and constipation or diarrhœa. Headache is more or less severe with sometimes delirium. In cases lasting for several days the skin appears slightly jaundiced. The urine is of the usual febrile type and, as a rule, shows the microorganism causing the disease. Death may occur in two or three days or within a week or in milder cases may be followed by recovery.

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* Prepared by Edward Fidlar.

After death from septicæmia the body tends to putrefy rapidly. The glandular organs all tend to be swollen and soft, especially the spleen, and parenchymatous degenerations are found to a greater or less extent. The lining membrane of the heart and vessels is blood stained, a rather characteristic feature of streptococcic septicæmia. Bronchitis and broncho-pneumonia are usually found.

Erysipelas is an inflammation of the skin, occasionally of mucous membranes, and the name is applied now only when the condition is brought about by streptococci. The inflamed area is very definitely outlined and may present blebs of a greater or less size. Œdema may be very marked where the skin covers loose tissue. Fever is present with its usual accompaniments. There may be vomiting, constipation or diarrhœa. There may be severe headaches or delirium. In fatal cases, death may occur without any apparent complication, or it may follow meningitis, pericarditis, nephritis or some other sequel. In simple uncomplicated fatal cases the liver, kidneys and spleen are swollen and soft and show degenerative changes in the gland cells.

Bronchopneumonia may be a primary condition or secondary to some other disease. The streptococcus produces a purulent inflammation in the terminal bronchioles and their surrounding alveoli. This lobular distribution may become practically lobar by the confluence of affected areas. Streptococcic bronchopneumonias are frequent in fatal cases of influenza.

Pasteur, Koch, Rosenbach and Fehleisen divide the earlier honors in the gradual working out of the relationships of streptococci to disease.

Blood culture in plain broth in the case of septicæmia or inoculation of plain nutrient agar from pus are practically always successful. Growth is never luxuriant on the ordinary media. Cultivation from cases of erysipelas is less easy because most of the organisms are found at the margin of the lesion and are difficult to reach.

In exudates a stained smear will usually demonstrate the chain-forming coccus at once.

The cocci vary in size from 0.4μ to 1μ. In shape the organisms may be rounded or oval or with one aspect flattened when occurring in pairs. The chains may be long or short and a grouping into pairs is frequent even within the chain. There are no true spores developed and the organism is non-motile. Capsules are not found on the majority of streptococci. Staining the organism is easily accomplished with the ordinary aniline dyes. It is Gram-positive. The temperature range in which streptococci are capable of growing is about from 15° to 45°, the optimum temperature is about 37°. Streptococci are, as a rule, aerobes and facultative anaerobes. Strict anaerobic species are said to have been isolated from fæces. The reaction of media should be slightly alkaline. Acid production is a striking feature of this organism and has a decided inhibitive effect upon its growth. Concerning the action on carbohydrates this organism typically forms acid from monosaccharides, lactose, saccharose, and salicin. Gas is never produced. Nitrates are reduced by some streptococci to nitrites. The production of hydrogen sulphide is characteristic of some forms which have been grouped as *Strept. fæcalis*. No pigment is found other than the slight brownish tinge seen in some gelatin cultures. Typically actively hæmolytic. This power may be lost on cultivation. The toxic products of the streptococci have been the subject of a great deal of investigation, but few definite facts have been discovered. When cultivated on plain nutrient agar the growth is visible in eighteen to twenty-four hours as small round translucent finely granular colonies, which possess an even or notched border, and a tendency to remain discrete except when thickly sown. The center is thickened and the margins thinner. In plain nutrient broth the majority of long-chained varieties produce at the bottom and along the sides of the tube a granular deposit, or small flocculi or large flakes, leaving the remainder of the broth clear. A few long-chained varieties cloud the broth uniformly. The short-chained streptococci, as a rule, produce a cloudiness in the medium which remains for a number of days even though a finely granular deposit accumulates at the bottom of the tube. On plates of plain nutrient gelatin the colony formation remains the same as that on agar. In stab cultures a finely granular filiform growth appears which later may have a beaded appearance and sometimes a brownish color. The gelatin is not liquefied. Milk is a favorable medium for the growth of streptococci and a strong acidity and coagulation sometimes takes place. Growth on potato is said not to take place, but in some cases an invisible growth seems to occur. Loeffler's blood serum is also a favorable medium. Streptococci, as a rule, die out rapidly in cultures due to the accumulation of their own products. In pus, blood, sputum, etc., the organism may be found alive after several weeks or even months at room temperature. The thermal deathpoint is about 54° in ten minutes. Direct sunlight kills within a few hours, and they are readily killed by many disinfectants.

Entrance of streptococci is afforded by any break in the surface of the body. A local suppuration may be the result or it may be followed by a general septicæmic condition.

In erysipelas some local injury is also probably necessary as a starting-point.

Following the local establishment of streptococci sufficient toxin is elaborated to produce greater or less systemic disturbance. If a septicæmia supervenes the poisoning becomes extreme and the organisms are distributed throughout the body.

Immunity following recovery from natural streptococcic infection is very slight if any, and never of a permanent sort. Septicæmias once established are generally fatal, and erysipelas can recur frequently.

Bactericidal substances, opsonins, agglutinins and precipitins have been demonstrated in immune sera, which, however, show little therapeutic success.

Streptococci are eliminated in the discharge of local infections in sputum, etc., and are then probably more virulent. Infection by contact from such sources is particularly dangerous. In anginas and streptococcic infections of the respiratory tract, the epidemiology is practically the same as for diphtheria and pneumonia. Similarly erysipelas is to be treated as a contagious disease.

In the prophylaxis of streptococcic diseases, greatest care must be shown where chances of infection by the virulent strains are possible. Isolation of erysipelas is universally practised in hospitals. Similarly cases of puerperal sepsis and any local disease should be kept from contact with other puerperæ. Streptococcic pus from all sources is to be carefully destroyed.

Streptococci seem to be always present on the exposed surfaces of the body and are probably capable of giving trouble should any local lowered resistance occur. The prevention of this may be accomplished by strict antiseptic treatment of wounds.

PNEUMONIA*

Streptococcus pneumoniæ

The occurrence of a diplococcus in the large majority of cases, especially of the lobar type of pneumonia, has caused this coccus to be regarded as practically specific and warrants the name of *Streptococcus pneumoniæ*, *Diplococcus pneumoniæ*, or *Pneumococcus*. As occasional causes of pneumonia should be mentioned *Streptococcus pyogenes*, *Staphylococcus pyogenes var. aureus*, *B. coli*, *Bact. diphtheriæ*, *Bact. influenzæ*, *B. capsulatus mucosus* (pneumobacillus), *B. typhosus* and *Bact. tuberculosis*.

Pneumonia is world-wide in its distribution and is estimated to form anywhere from 1 to 7 per cent of all cases studied in internal medicine. It appears to be more frequent in regions subjected to sudden changes of temperature and many more deaths occur in the five months December to April than in the remainder of the year.

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* Prepared by Edward Fidlar.

The incubation period is two or three days of rather indefinite prodromata.

The onset of the disease is marked by a chill, pain in the side, and rise in temperature. The respirations become frequent. The fever, as a rule, runs between 102° and 105°F. for from five to ten days and then in favorable cases terminates by a sudden drop of temperature to normal within a few hours (crisis).

The most striking pathological findings are a marked congestion and œdema of the lungs following which the lung becomes solid, airless and of a dark red color, the alveoli showing, microscopically, a fibrinous exudate with large numbers of red blood cells, some leucocytes and desquamated epithelium. Thereafter the lung becomes slightly softer and is of a gray color, while microscopically the red cells degenerate and leucocytes are much more in evidence. The final stage, resolution, is marked by the liquefaction and absorption of the contents of the alveoli and the entrance of air.

Death occurs from toxæmia or complications such as carditis, meningitis, etc. Roughly about 10 per cent of all deaths are due to pneumonia and the fatalities form about 10 per cent of the total number of cases.

The *Streptococcus pneumoniæ* was described, as found in the sputum, by C. Frankel in 1884.

A Gram-stained preparation of the sputum is sufficient to detect the diplococci but cultures are necessary for positive identification. Some medium richer than the ordinary by the addition of blood or serum from man or animals is best, and may be inoculated from the blood and organs or from sputum and other contaminated sources by streaking or plating. Injection of sputum into white mice or rabbits will often cause a fatal septicæmia in these animals and the coccus may then be obtained in pure culture from the heart's blood. It occurs as pairs of oval or lanceolate cocci, with their contiguous surfaces somewhat flattened and the distal ends slightly pointed. From this type the organism may vary to spherical or short bacillary forms. It may occur also singly or in chains of varying length usually consisting of not more than about six or eight individuals. Well developed capsules which may surround the single organism or the pairs and chains may be found in exudates or in milk and serum media. There are no spores nor flagella. The cocci stain readily with the aniline dyes and are Gram-positive. The capsule can be demonstrated by several methods of which Welch's and Hiss' are the most common. The temperature range is from 25° to 41°. It is both aerobic and anaerobic, and grows most readily in a medium slightly alkaline to phenolphthalein. Besides serum or blood, glycerin, nutrose and dextrose are found to be favorable for its growth. On agar it grows in small, rather transparent, finely granular colonies, which are larger and more opaque when serum or ascitic fluid is present. Broth is faintly and uniformly clouded. Milk is a favorable medium for most strains and typically is acidified and coagulated. On potato, growth may occur but is invisible. Gelatin can rarely be used at a temperature high enough to allow growth. When, occasionally, growth is obtained the medium is not liquefied. On blood serum, growth appears as small clear colonies and on the whole is better than on agar. A number of special media are described of which one of the most valuable is the inulin-serum-water medium of Hiss. It typically ferments, with the production of acid, the majority of carbohydrates, even polysaccharides as inulin. On blood agar the typical organism produces a greenish zone in the medium about the growth, but not a clear zone of hæmolysis as do most strains of streptococci. The differentiation from other streptococci is sometimes a matter of difficulty, and the following characters are of importance—the lanceolate shape, capsule formation, fermentation of inulin, absence of hæmolytic powers, agglutination in antipneumococcic sera, susceptibility to lysis by the action of bile salts. Acid is an important and characteristic product and, if allowed to accumulate, rapidly kills the organism. The toxic products appear to be closely united with the cell bodies and are only released when these are broken up. The resistance to heat is not great and its thermal death-point is 52°. Light is germicidal if the cocci are not protected in thick masses of sputum. Drying is resisted rather well in sputum or the blood of infected animals. To germicides the *Pneumococcus* is very sensitive and is killed in a few minutes by the common disinfectants in their usual strength.

The pathogenic properties of the *Pneumococcus* for animals is somewhat variable. Natural infection is not common. To artificial infection mice and rabbits have been found most susceptible, while guinea pigs, dogs, rats and cats are more resistant, and birds are practically immune probably because of their high body temperature. Mice are regularly used for the rapid isolation and determination of pneumococcus types. By special methods lobar pneumonia has been produced in rabbits as has also endocarditis.

Variations in virulence of the *Pneumococcus* are very marked. The virulence can be increased by passage through susceptible animals. In standardizing type sera, strains are used of which 0.000001 c.c. of a broth culture will kill a mouse.

The organism gains entrance through the respiratory mucosa and as a matter of fact appears to be a common inhabitant of these regions. However the organism may reach the lung (the lobar distribution suggests sowing by the blood stream), it is certainly frequent to find positive blood cultures during the disease—a fact which accounts for the development of such complications as meningitis, endocarditis, etc.

The toxæmia probably arises from lysis of the organisms and it has been shown that the autolysis of cultures in salt solution gives rise to a soluble toxic portion and an insoluble non-toxic portion.

Immunity to *Pneumococcus* infections can be shown to exist after an attack but only for a short time.

Pneumococci may be considered as inhabiting the mucous membranes of the respiratory tract in the majority of people and acquire virulence only under some special circumstances lowering the general vitality. In pneumonia and some kinds of bronchitis as above mentioned it should be remembered that sputum and mouth spray may contain large numbers of virulent organisms.

Specific therapeutic agents such as antipneumococcic sera, vaccines of dead cultures and autolysates, as well as leucocytic extracts, have been tried and all with some promising results. The earlier failures with serum therapy have been found to be due in part to the occurrence of different strains. Four strains or types are now recognized. About one-third of cases is due to Type I, one-third to Type II, 10 to 15 per cent to Type III and the remainder to Type IV. Under ordinary conditions the mortality of Type I and II is 25 to 30 per cent, of Type III 59 per cent, and Type IV 10 to 15 per cent. The corresponding antisera are more successful in Type I infections.

The prophylaxis of *Pneumococcus* infections lies in general hygienic measures, in the destruction of sputa and avoidance of possible infection by mouth spray, etc. In households in which pneumonias existed due to Types I and II, Stillman isolated the organisms from the dust in 43 per cent and 59 per cent respectively. Occasional carriers of Types I and II are found not associated with clinical cases. Type IV predominates in the mouths of the healthy.

ANTHRAX*

Bacterium anthracis

Also called splenic fever or charbon; and in man, wool-sorter's disease or malignant pustule.

The disease has been known for centuries. It is thought that it was one of the plagues of Egypt, mentioned as a murrain on beasts, and boils and blains on man and beast. The first accurate characterization of the disease was made by Chabert about 1800. Pollender in 1849 and Rayer and Davaine in 1850 reported that they had seen "filiform bodies" in the blood of animals which had died of anthrax, and in 1860 Davaine announced he had succeeded in transmitting the disease to healthy animals by inoculating them with blood from an anthrax infected animal, and asserted that these filiform bodies or bacteria were the cause of the disease. This result was attacked, and for ten years there was a fierce controversy over this idea, which was finally stilled by the convincing experiments of Robert Koch in 1876. Koch cultivated the bacterium of anthrax from the blood, showed that the inoculation of these cultures in susceptible animals produced anthrax, worked out the life history of the organism, and enunciated the cardinal requirements—which constitute the proof of the pathogenic nature of an organism, what later bacteriologists have named the rules or postulates of Koch.

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* Prepared by F. C. Harrison.

GEOGRAPHICAL DISTRIBUTION.—The disease is very widespread, occurring all over the world in tropic, semitropic, and temperate climates. Wherever stock are found in large numbers anthrax is usually present. The disease ravages the herds and flocks in Russia, Siberia, India, Argentina and parts of Hungary, France and Germany. Local epidemics occur constantly in England, Canada and the United States. In the delta of certain rivers the organism probably grows in the soil as in the deltas of the Mississippi and Bramaputra, and the disease is also common along the banks of many rivers (Vistula, Rhine, Seine, etc.).

The anthrax organism is a large, non-motile rod, from 5μ to 10μ long and 1μ to 1.5μ broad. In cultures it frequently forms long threads or filaments (Fig. 167). The free ends are slightly rounded, but those in contact are quite square, and slightly larger in diameter than the middle of the cell. Involution forms are obtained by culture on potato or at temperatures of 40° to 42°. It forms oval spores without distortion of the mother cell (Fig. 168). Free oxygen is necessary for the development of these bodies, and a temperature between 18° and 41°. Spore germination is polar. By culture at 42° an asporogenous variety is formed. It stains readily with the aniline dyes and also by Gram's method. Under certain conditions a capsule may be seen. The organism is aerobic, in the body it grows as a facultative anaerobe. Its optimum temperature is 37°, minimum 12°, maximum 45°. It forms characteristic wavy and filamentous colonies on gelatin and agar, it liquefies gelatin, produces an arborescent growth in gelatin stab cultures, coagulates and peptonizes milk with an alkaline reaction. Thermal death-point of the spores in liquids is four minutes at 100°, in hot air 140° for three hours. Mercuric chloride, 1 : 1000, destroys the spores in a few minutes, and 4 per cent carbolic acid with hydrochloric acid 2 per cent in one hour.

Zoölogically, anthrax is the most widespread of infectious diseases; white mice, guinea-pigs, rabbits, sheep, cattle, horses and man are susceptible. Old rats are insusceptible. Von Behring, Metchnikoff and others have shown that the serum of white rats contains a lysin capable of dissolving the bacterium *in vitro*. Pigs are occasionally infected; the carnivora generally are refractory, the bear and cat being less resistant. Most birds are insusceptible, but some small birds, like the sparrow, are more susceptible. Cold-blooded animals are refractory.

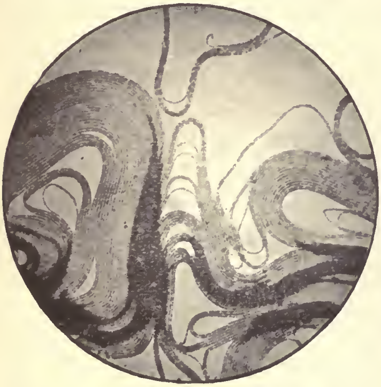

FIG. 167.—*Bact. anthracis*. Showing the thread formation of colony. (*After Kolle and Wassermann from Stitt.*)

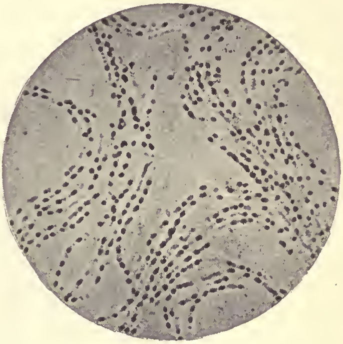

FIG. 168.—*Bact. anthracis*. Spore production. (*After Migula.*)

Infection occurs: Through the food, giving rise to intestinal anthrax. Cattle and sheep are usually infected in this manner by spores, the bacterium being destroyed by the gastric juice. In man infection through food rarely occurs.

Through the air. Infection by inhalation through the lungs occurs in man through the medium of dust contaminated by anthrax spores, hence the name "wool-sorter's disease."

Through wounds. This method usually occurs in man and also in sheep. Cutaneous infection comes through a scratch or wound, and gives rise to a carbuncle—hence the name "malignant pustule." It occurs most frequently among employees of tanneries, wool-sorters, veterinary surgeons, and those whose occupation brings them into touch with infected animals, their hides or products.

The incubation period is a short one, even in the naturally occurring disease; inoculated laboratory animals die in twenty-four to forty-eight hours. The bacteria appear in the blood about fifteen hours after inoculation, and at death the blood simply swarms with the organism. The veins are turgid, and the blood is often very dark, and coagulates slowly. The bacteria abound in the capillaries (Fig. 169). The spleen is enlarged and contains enormous numbers of the organisms. In the kidney the glomeruli and tubules are gorged with the bacteria, which pass into the urine. The bacteria can pass into the milk of females in lactation. The bacteria are also numerous in the liver, lungs and mesentery, but few are found in the muscles.

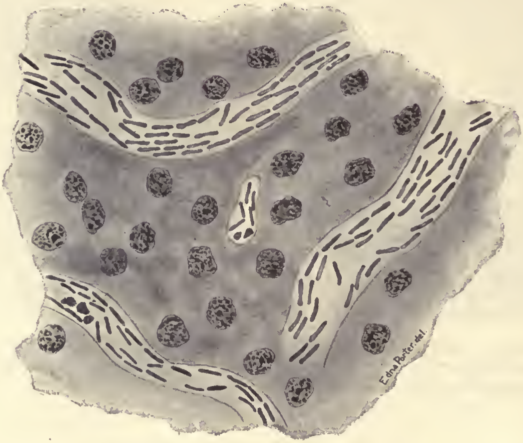

FIG. 169.—Anthrax. The organisms of anthrax in the capillaries of the liver of a mouse. (*After Williams.*)

Post-mortem examination of subcutaneously inoculated laboratory animals shows subcutaneous œdema and enlarged spleen.

The organism is eliminated from the body in urine, fæces, mucous discharges, etc. Pastures become infected from burying anthrax carcasses which have been opened or have been skinned, thus favoring the formation of spores. If buried too near the surface, the rise of the ground water, or the castings of earth worms, bring spores to the surface and on to the herbage, where they may be ingested by grazing animals. Tanneries using anthrax-infected hides may be the cause of distributing the organism by means of effluent water which has been used for steeping hides. Many such cases have been traced in Delaware, Wisconsin and in Ontario. Hay from an infected pasture may be transported to a distant farm, and cause an outbreak of the disease. In Brazil, vultures feeding on anthrax carcasses disseminate the spores by means of their excrement, and thus spread the disease. Blood-sucking flies may also be instrumental in transferring the bacterium from one animal to another.

Season is a contributing factor. In years in which the spring floods have been very high, followed by a hot dry season, anthrax is most prevalent.

There are a few preliminary symptoms; there is usually sudden loss of appetite, trembling and convulsive movements. Often blood is seen in urine or fæces or discharged from the nose. The mucous membranes are often bluish in color, and boils or pustules may occur on various parts of the body. Death in cattle occurs in two to five days and in sheep in twenty-four to thirty-six hours. The mortality is high and intestinal cases are fatal in 80 to 90 per cent of the animals attacked.

The usual post-mortem appearances are enlargement of the spleen, blood thick and tarry, bloody extravasations in the muscles and organs, and bloody fluids escaping from mouth, nostrils or anus.

In anthrax-infected districts vaccination should be used. The vaccines are prepared by cultivating the bacterium at a high temperature—42° to 43°—thus forming an asporogenous race, according to methods devised by Pasteur in 1881. Two vaccines are often used, the first of very low virulence, the second more virulent. Between 1882 and 1907, 8,000,000 sheep and 1,300,000 cattle have been vaccinated in France against anthrax, with excellent results. Vaccination by toxin has been advocated by Toussiant, Hawkin, Marmier and others, but this method has not had the success of that described above.

For treatment of the disease in man, Sclavo's serum has been of considerable benefit. This serum is obtained from the sheep or ass.

The animals first receive the two vaccines of Pasteur, then more virulent cultures in gradually increasing doses. A serum is then obtained which in a dose of 2 c.c. or less protects a rabbit against a lethal dose of the anthrax organism.

Animals dead of anthrax should never be opened or skinned. If doubt exists as to the nature of the disease, an ear may be cut off and sent to a laboratory for examination. Anthrax-infected carcasses may be either burned or buried at a depth of 1.8 m. (6 feet), and covered with quick-lime, and as an extra precaution the burial ground may be fenced off. The prime necessity is to prevent the formation of spores, as it has been shown experimentally that they remain in this condition for eighteen years and produce the disease when inoculated. Soiled litter, forage and the excretions of animals dead of the disease should be collected and burned.

The stalls, stables, implements and anything that has been in contact with the diseased animals should be disinfected by burning, boiling or the use of some disinfectant like 5 per cent carbolic acid.

Bacillary White Diarrhœa of Young Chicks*

Bacterium pullorum

The epidemic type of diarrhœa which is characterized in part by a whitish diarrhœal discharge, and which is now known as "bacillary white diarrhœa," is caused by a bacterium which belongs to the colon-typhoid group of organisms. It may be cultivated easily on the ordinary laboratory media, but its growth on slant agar containing Witte's peptone is delicate and bears a striking resemblance to that of *Streptococcus pyogenes*. This finely beaded growth is an important aid in the identification of the bacterium.

The specific organism, *Bact. pullorum*, is present in the liver, lungs, kidneys, spleen, heart and unabsorbed yolk of affected chicks, being most easily obtained from the liver and yolk, when the latter is present. Some of the most common post-mortem appearances of the organs are those of the liver and intestine, the former showing pale and congested areas, while the intestine is colorless and to a large extent void of contents.

The disease seldom manifests itself in chicks after they have attained the age of four or five weeks. The greatest mortality usually occurs within the first two weeks. The chicks become listless, and are inclined to huddle together for warmth. There is loss of appetite, and emaciation. The wings droop, the back seems to shorten and the abdomen protrudes out of proportion, causing the chicks to look stilty. The characteristic whitish discharge from the bowel may be absent from individual chicks, but is usually noticeable in groups of any appreciable size.

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* Prepared by L. F. Rettger.

Bacillary white diarrhœa may be transmitted to young chicks under five days old through infected food and drinking water, as has been demonstrated repeatedly. Furthermore, chicks are often infected with *Bact. pullorum* before they are hatched. This is due to the fact that the yolk of infected hens carries the specific organism in it from the time of its formation in the ovary. Hence, the mother hen is the source of infection, having retained within it the bacterium in question from the time she was an infected chick, or having acquired it later in life through contact with diseased fowls. In laying hens the infection is localized in the ovary which becomes decidedly abnormal in appearance. The partly developed ova are discolored, misshapen and of all degrees of consistency.

Ovarian infection may be determined by the macroscopic agglutination test which has proven itself very valuable and practicable in the organized campaign against bacillary white diarrhœa that has been conducted in the State of Connecticut for the past six years. This method of diagnosis has been found to be much more valuable than the bacteriological examination of eggs.

Eradication of infected laying stock is the solution of the white diarrhœa problem. Flocks which are at all doubtful, or which have given a history of infection, should be tested, and the reacting fowls eliminated. Better still, no eggs should be used for hatching which have come from flocks that have shown an appreciable degree of infection, although reacting individuals have been removed.

CHICKEN CHOLERA*

Bacterium choleræ gallinarum

The bacterium causing this disease was first noticed by Perroncito and Toussaint; later, in 1880, it was described by Pasteur, and was the first organism in which the French savant succeeded in attenuating the virulence and the first disease for which a vaccine made from attenuated organisms was prepared. Koch in 1878 described an organism of similar pathogenicity as the bacterium of rabbit septicæmia and in 1886 the term hemorrhagic septicæmia was given by Hueppe to a number of infectious diseases of the lower animals in which hemorrhagic spots were found in the tissues and internal organs. In 1900 Lignières discussed these bacteria, and named them as a genus, *Pasteurellose*, the specific name given depending on the animal for which it was most pathogenic. Thus he distinguished avian, porcine, ovine, bovine, equine and canine *Pasteurelloses.*

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* Prepared by F. C. Harrison.

The specific characters of this group are small ovoid bacteria, often showing bipolar staining when treated with the aniline dyes, non-motile, no spores, Gram-negative, polymorphic, not liquefying gelatin, no visible growth on naturally acid potato, milk unchanged, no indol production, generally aerobic but also a facultative anaerobe, virulence changeable, but usually very pronounced.

The bacterium of fowl cholera, *Bact. choleræ gallinarum,* or avian *Pasteurellose,* is from 0.5μ to 1.25μ long and 0.25μ to 0.40μ broad. It develops best at 37°, and very slowly at 20°. It loses its virulence in cultures very quickly, and it succumbs readily to desiccation.

The disease is of frequent occurrence in Europe, but not often seen in North America but some outbreaks have been reported in the United States and Canada. Unfortunately it has been confused by poultrymen with any disease characterized by excessive diarrhœa. The symptoms first noticed are the yellow color of droppings soiling the cloacal feathers, then diarrhœa sets in, the character of the discharge varying, being at times a fluid greenish mass, or a brown-red mucus, or a viscous transparent and frothy fluid. The bird becomes uneasy, drinks copiously and with a rise in temperature to 42° to 44° the bird becomes drowsy and death follows. The period between the first noticeable symptoms and death varies from one to three days. Chronic cases sometimes occur and in these the bacterium is found with difficulty. The birds become infected by way of the digestive tract, from eating and picking up material infected by the discharges of diseased birds.

Post-mortem indications are blackened combs, congestion of the blood-vessels in the organs and intestines, and punctiform or large hemorrhages of the duodenum, intestines and heart. The bacteria are numerous in the blood, the pulp of all organs, and in the intestinal contents. It is a true septicæmia.

If the disease breaks out in epidemic form the best and quickest method of getting rid of it is to kill off all the fowls, disinfect the houses, and dig or plough up the poultry runs, and leave them two weeks before re-stocking.

CHRONIC BACTERIAL ENTERITIS*

Bacterium panatuberculosis

The disease produced by this bacterium has been demonstrated in Germany, Belgium, Switzerland, Holland, Denmark, and perhaps other European countries. It is known by various names, as *Johné's disease, chronic bacterial dysentery*, and *chronic bacterial enteritis.*

This bacterium produces a chronic infectious disease of cattle involving especially the intestinal mucous membrane and related lymph glands. Other animals do not seem susceptible. The disease produced is usually fatal. Usually the most conspicuous general symptom is unthrift in spite of good appetite and good food and a chronic incurable diarrhœa.

This microörganism is a rod-shaped bacterium from 2μ to 3μ long and about 0.5μ broad and is strongly acid-fast. The production of active toxins is to be presumed since the amount of disturbance is frequently out of all proportion to the lesions found on examination post-mortem. The period of incubation has not been defined, but is apparently very long.

The bacteria are present in the fæces, intestinal mucosa, and submucosa, most frequently in the small intestines. The large intestines may be involved later.

This microörganism produces chronic, inflammatory changes of the intestinal mucous membrane, the whole intestinal wall becoming greatly thickened.

This bacterium resembles closely avian tubercle bacteria, but may be distinguished by the fact that the avian tubercle bacterium is rather easily grown on artificial media. This organism does not have the same pathogenic peculiarities as the avian tubercle bacterium. It seems well demonstrated that many cases of *chronic bacterial enteritis* do probably react to avian tuberculin; but this does not prove identity.

So far as known the bacterium is eliminated in the manure of affected cattle and disseminated in this way. Wider dissemination is made by diseased animals moving from place to place.

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* Prepared by M. H. Reynolds.

The most important considerations in controlling this disease are careful disposition of contaminated manure and isolation of suspected animals. The manure should be used only where it can not serve to spread disease to other cattle. Sick animals should be carefully isolated and premises thoroughly disinfected.

CONTAGIOUS ABORTION OF DOMESTIC ANIMALS*

Bacterium abortus

The premature discharge of the products of conception from the uterus is a not infrequent occurrence among domestic animals, and doubtless various factors may from time to time operate in its causation. Injury, excessive fermentable food, or poisonous food may at times produce this result. For a long time, however, practical husbandmen have recognized an epizoötic form or a contagious abortion, a definite transmissible disease, of which the loss of the fœtus is the most prominent characteristic. This disease appears to be generally distributed in all agricultural communities. Cows, especially, are affected, but a somewhat similar if not identical disease also occurs in other domestic animals.

In 1897 Bernhard Bang discovered in the uterine exudate of a cow, slaughtered during an attack of this disease before the abortion had occurred, a small bacterium which he was able to grow in pure culture, and, by inoculating pure cultures of this organism, he produced the disease in cows, sheep, goats and rabbits.

The microbe is a short non-motile rod, staining with moderate ease, and decolorized by Gram's method. It does not form spores but the vegetative forms are fairly resistant to drying and may, perhaps, live for some weeks under ordinary conditions in pastures and stables. Its artificial culture requires special technic because of its peculiar oxygen requirement. The bacterium usually fails to grow in the presence of the atmospheric air or under anaerobic conditions. It requires for its development a partial pressure of oxygen somewhat less than that of the atmosphere. When inoculated into deep serum-gelatin-agar tubes and incubated in the air, the colonies develop only in a particular zone about five millimeters beneath the surface of the medium. When cultures are placed in the proper atmosphere, development on the surface may be obtained. Prolonged cultivation on artificial media obscures this peculiar property of the microbe so that old culture strains grow well under ordinary aerobic conditions.

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* Prepared by W. J. MacNeal.

In the diseased animal, the specific bacteria are found in the placenta and amniotic fluid, frequently also inside the fœtal intestine, sometimes in the tissues of the fœtal organs, and in the wall of the maternal uterus. The placenta appears to be the particular organ favorable to the development of the germ, and when this has been discharged from the body the abortion bacilli no longer flourish, although the infection may continue as a chronic uterine inflammation for a long time. The general health is only slightly disturbed. At the next pregnancy the disease is practically certain to reappear, and possibly again also at the succeeding one. After two or three abortions the animals appear to have acquired an immunity to the infection, and may sometimes breed normally thereafter, although some animals are permanently sterile after a few attacks of the disease.

The organisms escape from the diseased animal in the products of conception at the time of the abortion, and in the chronic uterine discharge which may continue for a long time afterward. The disease may be conveyed to other animals by contact with this material and by eating grass or other feed soiled with it. Doubtless the male is an important factor, possibly the most important factor, in transmitting the disease, although no serious inflammation is produced in him.

The control of the disease depends upon the isolation of the infected animals, cremation of infected fœtus, placenta and discharges, and thorough disinfection of the premises. Heifers and healthy cows should not be allowed to mingle with cows which have aborted, nor should they be served by a bull which has covered infected animals at any time. Local antiseptic treatment of the cow which has aborted diminishes the danger of the persisting discharge.

Contagious abortion also occurs in other domestic animals, especially in horses, sheep, goats and swine. Inoculation experiments have shown that the *Bact. abortus* of Bang can infect some of these animals. Its importance as a factor in the epizoötics of abortion occurring naturally among them is still uncertain. In horses at any rate another organism appears to be more frequently involved.

DIPHTHERIA*

Bacterium diphtheriæ

The disease is epidemic in all large communities especially in Europe and America. It is, however, almost absent from tropical regions. Epidemics and pandemics occur in cycles. Essentially diphtheria is a disease peculiar to man. Avian diphtheria, however, is known, although seemingly due to another cause, and on rare occasions natural infection has been found in the horse.

The period of incubation is said to be two to five days.

In man the disease usually begins with lassitude and fever followed in a few hours by "sore throat." The inflamed area on the pharyngeal wall, tonsils, larynx or wherever it may be becomes in typical cases the seat of degenerative changes in the epithelium and underlying tissues with abundant fibrinous exudation resulting in the formation of a comparatively tough membrane or pseudo-membrane, which is a striking and characteristic feature of the disease. This local lesion is almost always found on mucous membranes though occasional instances of infection of wounds have been noted.

In connection with wound infection it should be mentioned that organisms morphologically resembling the true *B. diphtheriæ* occur upon the body surfaces and are commonly called "diphtheroids." They are of some importance because occasionally they have to be distinguished from *B. diphtheriæ* by culture and animal inoculation, and secondly, while of doubtful pathogenicity, they are held by some to be responsible for a certain indolence in the healing of wounds. In an investigation by Col. Adami and others in the Canadian army it was found that in some localities as many as 33 per cent of war wounds were infected with diphtheroids of at least four different types, while true *B. diphtheriæ* occurred in less than 0.6 per cent.

The bacterium of diphtheria was described in 1883 by Klebs in sections of typical membranes. The organisms were isolated and differentiated in 1884 by Loeffler, who was able to fulfill Koch's postulates for pathogenic microbes. Accidental infection of the human being has happened in the laboratory and confirmed the findings of animal inoculation. The success of antitoxin treatment is further evidence of causal relationship.

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* Prepared by Edward Fidlar.

The organism is detected in the following manner: A sterile swab is rubbed gently over the inflamed area or against any visible membrane, care being taken to touch other parts as little as possible. The swab is then immediately inserted into a tube of specially prepared medium—Loeffler's inspissated blood serum—over the surface of which it is rubbed back and forth. The swab is returned to its own tube or left against the serum and the culture and swab sent to the laboratory. After twelve to eighteen hours of incubation, at 37°, smears are made from the cultures and stained with Loeffler's methylene blue. The diagnosis is made on the morphological characters of the bacterium. Occurring in pure cultures the form of the bacterium is subject to remarkable changes according to the medium and length of cultivation. Its size as it appears in exudates and from serum media varies from 1μ to 7μ in length and 0.25μ to 2μ in width. The rods are straight or slightly curved, usually not uniformly cylindrical but with swellings at the ends, or in the middle, or irregularly disposed (Fig. 170). Both ends may be rounded or both pointed, or one rounded and the other pointed. Branching forms are not infrequently found. The bacteria may appear in pairs end to end; more frequently and typically they are inclined to one another at a greater or less angle and may assume a parallel arrangement or the form of a short zigzag chain. The arrangement is most clearly understood and remembered by considering the peculiar "snapping" type of fission characteristic of the group. There are no flagella and no spores. The cell membrane is possessed of great elasticity as evidenced by the post-fission movements. The bacteria stain readily with all the aniline dyes and retain the primary stain fairly well by Gram's method. With Loeffler's methylene blue they stain in a characteristically irregular manner, and show metachromatic "granular" forms, "barred"

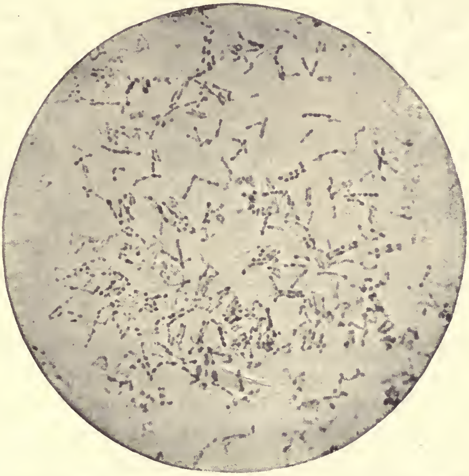

FIG. 170.—Bacterium of diphtheria. × 1000. (*After Williams.*)

and "solid-stained" forms (Fig. 171). On a basis of morphology and staining properties, Wesbrook, Wilson and McDaniel have devised a classification which is very convenient for descriptive purposes. The minimum temperature of growth 18° to 19°, optimum 35° to 37°, maximum 40° to 41°. The bacterium grows most readily in the presence of oxygen. Under certain conditions it will grow anaerobically. The optimum reaction of blood serum media is about +0.8. The amount of acid which the bacterium can endure varies with the kind of acid. Gelatin is not liquefied, neither are the proteins of blood serum nor of milk. Caseinogen is not changed to casein. Some carbohydrates are broken up with the production of acid. All authorities find that the bacterium forms acid from dextrose. It is generally agreed that acid is produced from lactose, galactose and maltose. Action on dextrin, lactose, saccharose and glycerin is variable. The majority of workers find mannit is unchanged. An acid reaction in plain broth by fermentation of muscle sugar may be followed by the production of alkali. Gas is not produced under any circumstances. No indol is formed. Most strains cause hæmolysis of red blood cells. A true diffusible toxin is formed for the artificial production of which in broth cultures peptone, absence of sugar, an alkaline reaction and free access of oxygen are favorable factors. Growth on plain nutrient agar is not so abundant as on Loeffler's blood serum. Colonies of two types may be found: (*a*) most common is small grayish-white, rounded, slightly raised, almost translucent with more or less granular surface and dark center, the margins varying in irregularity, and often with a thin extension spreading out from the edge; (*b*) less common, larger, more luxuriant, white, rounded, raised, granular to nearly smooth and somewhat moist. Plain broth must be slightly alkaline to litmus. About one-half of cultures grow readily and half very feebly. The characteristic growth is a finely granular deposit at bottom and along sides of the tube leaving the broth clear; a

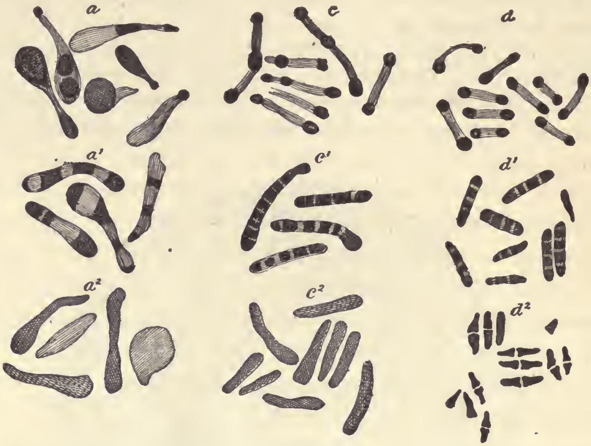

FIG. 171.—Wesbrook's types of *Bact. diphtheriæ.* *a*, *c*, *d*, granular types; *a'*, *c'*, *d'*, barred types; a^2, c^2, d^2, solid types. × 1500. (*From McFarland.*)

few cultures produce a diffuse cloudiness with more or less well-developed pellicle. Growth on gelatin is scanty chiefly owing to temperature at which this medium must be kept. The gelatin is not liquefied. In milk growth takes place at comparatively low temperature (20°) without coagulation but with acid production. On potato growth occurs especially if slightly alkaline; in the majority of cases invisible; it may appear as a thin dry glaze or with a whitish or slightly yellowish tinge. On Loeffler's blood serum growth is rapid. In eighteen to twenty-four hours colonies are rounded, grayish-white with a slightly yellowish tinge moderately translucent except toward the center, smooth, moist and shiny. The margins are only slightly irregular. With age the colonies become dull and opaque, the surface becoming marked by concentric lines and sometimes also exhibiting radial striation. Thermal death-points are 58° to 60° for ten minutes, 70° for five minutes, 100° for one minute. On the other hand −190° for seven days and −252° for ten hours have failed to kill in some instances. Diffuse light hinders growth. Direct sunlight kills within two hours to a few days according to the medium in which the organisms are suspended.

The organism gains entrance into the body through the mouth and nose.

The bacteria usually remain localized; they can almost always be demonstrated when a definite membrane is present and often when there is none. They are practically always found in the lung of fatal cases because of direct infection. Entrance of the bacteria into the blood stream with resulting infection of the internal organs has occurred in fatal cases.

The protective apparatus concerned in diphtheria is probably different at the beginning from that involved late in the disease. Experimentally, agglutinins, bacteriolysins and opsonins have been demonstrated in exudates and serum. While these properties may be important in warding off an infection they appear to be of little influence once the bacteria are established, and thereafter on the amount of antitoxin will rest the outcome of the disease.

The toxin is strongly antigenic, the cell bodies feebly so. Aggressins have not been demonstrated.

The organisms escape by the secretions of the mouth and nose. Direct infection by coughing, sneezing and speaking probably takes place frequently not only from the sick and convalescents but also from healthy carriers.

Control of the disease is sought by quarantine of all sick persons and the placing of restrictions if not actual quarantine on those exposed and showing the bacteria in the nose and throat

DYSENTERY*

Bacterium dysenteriæ

Two chief types of dysentery are known, one due to a protozoön, *Entamœba histolytica;* the other due to a bacterium—*Bact. dysenteriæ.* Only the latter is here dealt with.

Acute dysentery in an epidemic form is found chiefly in Asia, sometimes in Europe and in the Philippines. In this country occasional small epidemics and certain types of summer diarrhœas of infants have been shown to be due to *Bact. dysenteriæ.* The disease occurs naturally only in man.

Dysentery is an intestinal disorder usually acute and, in its epidemic form, very severe, marked by a flux in which there is the frequent passage of blood and mucus with severe tenesmus and pain in the abdomen. The fever accompanying this may reach 104° and in the severe cases delirium and death may result. In Japanese epidemics the fatality has reached 25 per cent or more.

The pathological findings are most marked in the intestine where the mucosa is swollen and hyperæmic, with more or less hæmorrhage and extensive necrosis.

Shiga in 1898 discovered a bacterium in the stools of persons suffering from the disease and the organism was agglutinated by the blood serum of the patients. He found the same organism repeatedly in a considerable number of cases.

The results of many other investigations have demonstrated the presence of several forms conforming in general to the type described by Shiga but showing some difference in fermentation properties; these are sometimes known as para- or pseudo-dysentery bacteria. The most important of these are the Flexner, Strong, and Hiss' and Russel's "Y" types.

The constant presence of the organism in the epidemic type and the fact of agglutination leave little doubt as to the etiological relation. A criminal fed with a pure culture of Shiga's organism developed the typical disease.

The organism can occasionally be isolated in almost pure culture from bits of mucus in the stool. Endo's and other special media may be used for isolation, but ordinary litmus lactose agar plates are satisfactory for Shiga and "Y" types.

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* **Prepared by Edward Fidlar.**

Bact. dysenteriæ (Shiga) is rather short with rounded ends and closely resembles the typhoid bacillus morphologically except that it does not possess flagella.

It stains readily with the aniline dyes and is Gram-negative. It grows best at body temperature, is aerobic and facultatively anaerobic. It prefers a slightly alkaline medium. On agar, broth, and gelatin growth resembles that of the typhoid bacillus. In litmus milk an alkaline reaction usually follows a slight primary acidity without any further apparent change. On potato growth it is at first invisible but may appear later of a brownish color. Acid is formed from dextrose, levulose, and galactose. (Other types described differ from this in the fermentation of mannit and sometimes of maltose.) Gas is never formed. Indol is not formed. (Other types usually form indol.) The toxins are probably chiefly endotoxins, though soluble poisons have also been demonstrated by some workers. The bacterium remains alive for months when preserved under the proper conditions. The thermal death point is 60° and resistance to low temperature is considerable. It is sensitive to the usual strength of ordinary disinfectants.

Dysentery does not occur in animals under natural conditions. By artificial methods, however, it is claimed the disease has been reproduced in dogs. Cultures, living or dead, are often extremely toxic to small animals, especially the rabbit, and produce, after intravenous injection, violent intestinal symptoms, due evidently to the excretion of an irritating poison. Nervous symptoms are also more or less marked and paralysis sometimes occurs before death. Immunity produced artificially in animals is accompanied by the production of lysins and agglutinins and lately antitoxins have been described in accord with the demonstration of diffusible toxins. The agglutination in man is of diagnostic value.

The epidemiology of dysentery is the same as for typhoid fever. In a few instances the bacilli have been demonstrated in the fæces of healthy persons, and convalesce may remain carriernts for several months.

Some success has been recorded from the administration of animal immune sera and has been attributed to both lytic and antitoxic action. Active immunization as a means of prophylaxis does not seem to be of much value.

Fowl Diphtheria*

This disease, popularly known as Roup, and in its later stages canker, is characterized by a grayish-yellow fibrinous exudate, called a false membrane, which forms upon the mucous membrane of the eyes, nasal passage, mouth, pharynx and larynx.

Roup, or fowl diphtheria, may be caused by a number of different organisms, among them the well-known *Ps. pyocyanea* (green or blue pus organism), *B. cacosmus* and other species which give rise to a complex suppurative process. The pus formed is semi-solid, cheese-like and yellowish-white in color without any tendency to become soft and liquid or to perforate the surrounding skin. The organisms have a tendency to penetrate the deeper layers of the mucous membrane or sub-mucous tissues, and hence swabs or cultures taken from the false membranes may not contain the causal microörganisms which are retained in the depths of the tissues. Sections of membranes from affected fowls show large numbers of pus cells, some regular masses, débris of epithelial cells and bacteria, and thus they differ from the diphtheritic membranes of man.

The organisms mentioned above have been isolated from affected fowls, not only in America but also by Hauser in Europe. Several investigators have described other bacteria producing false membranes, and there are a few who think that coccidia are associated with the disease.

Both *Ps. pyocyanea* and *B. cacosmus* are able to produce false membranes when inoculated into healthy birds, typical croupous and diphtheritic membranes in the mouth and eyes; tumors in the subcutaneous tissues, the contents of which are firm, cheesy and yellowish-white; purulent conjunctivitis, blindness, purulent ophthalmia, and cheese-like exudations in the bronchial tubes. These indications are identical with the symptoms of "roup."

The disease is of variable virulence, and is apt to become chronic, especially in unhygienic surroundings, and in draughty, badly ventilated damp houses. A common cold is a predisposing factor, and favors the invasion of the organisms mentioned.

Treatment of severe cases is useless, and demands too much time. Diseased birds should be isolated and the buildings thoroughly disinfected. Slight cases may be cured by a 2 per cent solution of potassium permanganate, in which the bird's head is plunged for a few seconds. This treatment should be given twice a day and continued until all symptoms have disappeared. The most effective preventive is to keep fowls in good sanitary conditions—in dry, clean and well-ventilated houses, free from draughts.

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* Prepared by F. C. Harrison.

Besides the organisms mentioned, Loeffler has described the *B. diphtheriæ columbarium,* and Loir and Duclaux the *B. diphtheriæ gallinarum* as causing fowl diphtheria, but the diseases produced by these organisms are very dissimilar from the well-known "Roup" of North America. The Klebs-Loeffler bacterium of human diphtheria has no pathogenic effect on fowls.

GLANDERS*

Bacterium mallei

Glanders is a very common and serious disease, most common among equines. It is communicable to the human being by inoculation and by the same process may affect sheep, goats, and laboratory animals. Cattle are not susceptible.

Bact. mallei and the disease it produces are widely scattered over the civilized world wherever horses are numerous.

This infection produces a disease which may be acute or chronic according to the virulence of the microörganisms and resistance of the animal. Mules and donkeys are less resistant than horses and usually have the disease in more acute form.

The characteristic features of the disease produced are inflammatory changes of the lymph glands and lymph vessels, ulceration of mucous membranes, the tubercle, the farcy bud, the lymph cord, and the peculiar, clear, viscid discharge. There is considerable fever in acute cases, much less marked or absent in chronic cases. In a very common type of the disease there frequently occurs a destructive inflammation of the nasal mucous membrane which results in ulcers and consequent nasal discharge.

Glanders in man is rare considering the frequent opportunity for infection. There are usually inflammatory swellings with involvement of local lymph glands, and constitutional disturbances soon follow the local symptoms. Human glanders is to be always regarded as very serious with a probability of fatal termination. Ulcers may develop in the nose or mouth with more or less discharge. Pustules appear involving the skin, and abscesses involve deeper structures in various portions of the body.

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* Prepared by M. H. Reynolds.

The distribution of *Bact. mallei* in the animal body is shown by the most common appearance of its disease in the skin, subcutaneous tissue, mucous membranes, lymphatic system, lungs, liver, spleen and kidneys.

The etiological factor is a small bacillus with rounded ends known as *Bact. mallei,* discovered by Loeffler and Schütz and several others in 1882, and well demonstrated to be the specific cause of glanders.

Entrance is usually effected by way of a mucous membrane, frequently the intestinal, sometimes by inoculation. The period of incubation seems variable and uncertain under natural infection, but in artificial inoculation with virulent cultures, is very brief.

Bact. mallei produces toxins in artificial media and also in body tissues; the well-known preparation called *mallein* may be considered in this class awaiting more definite knowledge. This substance produces a distinct reaction by inoculation into glandered animals, but is practically non-toxic for healthy equines. So far as known *Bact. mallei* attacks the animal tissues as do many other microörganisms, the harm resulting chiefly from bacterial toxins which give the local tissue reactions leading to the lesions characteristic of glanders.

In its action on tissues *Bact. mallei* resembles *Bact. tuberculosis;* but shows a more rapid development of lesions and more active inflammation.

Lesions are of two types—a well-defined nodule followed by ulceration and areas of diffuse infiltration.

The nodule as it appears in glanders consists largely of lymphoid cells and connective tissue, the latter increasing as the case becomes chronic. Nodules die at the center, suppurate, and discharge. This occurs especially in the external form of glanders, which affects more commonly the legs and head. Occasionally defined enlargements appear in the involved lung areas. Pulmonary lymph glands are frequently enlarged, and hardened. The superficial skin lesions are in the form of nodules previously mentioned, which usually suppurate and ultimately heal. In the deeper subcutaneous tissues there is a tendency to abscess formation. Small nodules or tubercles commonly appear in the lungs of affected horses. These vary in size from millet seed to as large as garden peas. Various degrees of broncho-pneumonia appear and more or less pleurisy.

Bact. mallei shows no flagella and is non-motile. It is a small bacterium 0.25μ to 0.4μ thick by 1.5μ to 3μ long with rounded ends (Fig. 172). Spores have not been demonstrated. It is generally single. Coccus forms sometimes appear and even short threads when grown on certain media; *e.g.*, potato. It decolorizes by Gram's method and is not easily stained by aqueous, alkaline aniline dyes. This bacterium grows fairly well between 25° and 42° on potato, glycerin agar, or blood serum. The guinea pig gives a reliable diagnosis by inoculation, showing a diagnostic reaction within four or five days. Diagnosis may also be confirmed by the agglutination test in dilution of about 1 : 800 or more and by the complement fixation test. Satisfactorily stained in tissue section by Kuehn's carbol-methylene blue. Its growth is limited at an upper range of about 42°. *Bact. mallei* is difficult to isolate by culture methods being a slow grower and easily lost beside faster growing organisms. It can be better isolated by guniea-pig inoculation. In growth it is both aerobic and anaerobic, but better under aerobic conditions.

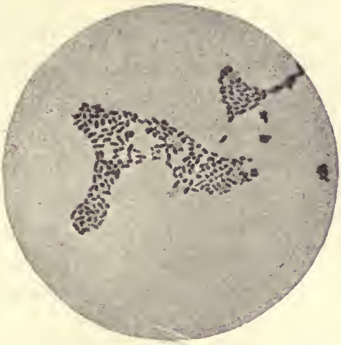

FIG. 172.—*Bacterium mallei.* From pure culture on glycerin agar. × 1000. (*From Migula.*)

The virus escapes from the body in various ways. Elimination is most common in morbid discharges from the nose, pharynx, trachea, and in pus from farcy buds and abscesses.

Bact. mallei may be spread directly from the diseased animal to the susceptible animal, or the dissemination may be by way of intermediate objects; *e.g.*, troughs, feed boxes, water pails, etc. It is easily killed by drying, sunlight, disinfectants, and heat; but may remain alive two years or more in water under favorable conditions. Heating to 55° kills in about ten minutes.

In man, infection occurs usually by inoculation. Cases produced in this way, occasionally appear among laboratory workers.

All plain cases of glanders in domestic animals should be promptly destroyed. Exposed horses should be tested with mallein. Those that react should be destroyed or quarantined, and contaminated premises properly disinfected. Immunization has not been satisfactorily established. Diagnosis of doubtful cases is made by the use of mallein.

INFLUENZA*

Taking influenza with its complications, the recent pandemic in its dimensions and the swiftness of its movements has been one of the most remarkable diseases in the annals of medicine. Reliable and complete statistics are not yet available, but undoubtedly the world as a whole will have to record deaths by the millions and cases by the hundreds of millions, and these chiefly within the year 1918. Its widespread occurrence, the mystery of its cause, its startling infectiousness, and its later high mortality formed a combination which in psychological effect gained it a place nearly comparable with an old time pestilence.

It has seemed at times as though the public felt a grievance against the medical profession for its comparative ignorance of the disease. Several difficulties, however, have attended its investigation. Local outbreaks so suddenly appeared and so rapidly declined that there was insufficient preparation for their study, or where preparation was adequate the cases would disappear before a definite line of research could be followed to conclusion. It was unfortunate, too, that with the doubtful exception of the monkey, no experimental animals were available to carry on the work. Finally, it has been difficult to determine what constitutes pure influenza, and whether, in the very fatal pneumonias which latterly appeared with it, the associated organisms played a major or a minor part.

Evidence is accumulating that the bacterium of Pfeiffer described below and still known as *B. influenzæ* is not the cause of the disease, but takes the rôle of a secondary invader similar to that of the pneumococcus and streptococcus. There is evidence supporting the theory of a filtrable virus; a bacillus of the hæmorrhagic septicæmia group has been described as a probable cause; various cocci and a symbiotic combination of organisms have also been suggested. At the present writing the specific infective agent must be regarded as unknown.

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* Prepared by Edward Fidlar.

Some question the identity of the recent influenza with the former disease of the same name epidemic in 1889 to 1892, but the term has been rather loosely used in the interval, and it seems probable that we have had a recurrence of the original disease but in more virulent form.

Spain is given by some as the place of origin of this last outbreak (whence the term "Spanish Flu"), and, if true, it seems worthy of note that, after a quiescence of twenty years, the epidemic revived in a neutral country rather than in a war-swept country where some sections of the population were living under abnormal conditions of nutrition and sanitation. Once started, however, war conditions certainly hastened its spread, testified by the explosiveness of some of the outbreaks in the armies where the intimate contact of large groups of men was so much greater than in civil life. The swift passage from person to person thus afforded to the specific virus and its concomitant organisms may to some extent explain the remarkable virulence finally attained.

The natural disease probably occurs only in man. Reports of epidemics among animals have not yet had scientific confirmation, and the experimental disease in the monkey needs further study.

The incubation period varies from about one to seven days.

The milder type of influenza which occurred in the spring of 1918 both in Europe and America, seems more likely to have been the pure infection than the more fatal kind appearing in the following autumn and winter. The initial stages were practically the same in the two types, but in the winter type signs of lung involvement developed with greater or less rapidity.

The onset is sudden, with headache, pains in the back and limbs, chilliness, dry throat, suffusion of the face and conjunctivæ, and occasionally nose-bleed. The temperature will range from 100° to 104°F. within a few hours but the pulse remains relatively slow; in many groups of cases the blood-pressure has been uniformly low. The leucocyte count is below or at normal with a relative increase of lymphocytes. This may give place later to a total and neutrophilic increase as pneumonia develops. Cough may be absent at onset but usually develops, dry at first and later productive as pneumonia sets in. Herpes sometimes occurs. The prostration and depression of influenza are a characteristic feature. After three to five days in the milder types, the temperature comes to normal by lysis, but convalescence as a rule is slow. Cases developing pneumonia are usually marked by cyanosis a secondary rise in temperature, a change in the sputum from a scanty mucoid to a purulent and often blood-stained character, an increase in the respiratory rate, and the appearance of varying physical signs in the chest.

The most noticeable pathological findings in fatal cases occur in the chest. Generally speaking, there is a very moist, confluent, lobular pneumonia showing hæmorrhagic zones together with firmer yellowish areas large or small, sometimes lobar in extent and occasionally showing fibrin. The cut bronchioles yield a thick yellowish pus and often appear as centers of greater or less necrosis. This lesion suggests that of the purulent bronchitis described by English writers in 1916 and 1917 but of more advanced character and with an added pneumonia. A tracheo-bronchitis is present which is usually hæmorrhagic. Hæmorrhage in the abdominal recti is found in some of the severe cases.

There is no doubt that the specific organism enters and leaves the body by the mouth and nose.

It seems probable that an immunity following the disease may endure for at least a few months, but it is difficult to secure data for longer periods.

No specific substances are known for the treatment and prevention of the disease except that prophylactic vaccination against the secondary organisms such as *B. influenzæ*, the pneumococcus and streptococcus, etc., have been tried with reported success both in England and America. The mask has probably been an aid against the spread.

Bact. influenzæ

This organism was described by Pfeiffer in 1892 as occurring in large numbers in the purulent bronchial secretion expectorated by influenza patients, and until the present pandemic was regarded by many as the established cause of the disease. In pure cultures the bacterium is 0.2μ wide by 0.5μ long with occasional threads up to 2μ in length. Larger forms are seen on boiled blood agar. The arrangement is usually single, occasionally in pairs end to end and rarely in chains. The bacterium is non-motile and does not show spores or capsules. It does not stain very readily, sometimes shows polar staining, and is Gram-negative. The temperature range is about 26° to 41°C. It is aerobic. Hæmoglobin is usually regarded as an essential constituent of media for its growth. Superheated or boiled blood agar affords more luxuriant growth than agar containing unaltered blood. Colonies are small, round, and transparent and remain discrete unless thickly sown. Growth occurs in blood broth used in thin layers. Resistance is less than the majority of non-spore-bearing bacteria. It seems especially sensitive to drying and its thermal death point is 60° C. for about one minute. The bodies of the bacteria are distinctly pyogenic. Inoculated animals develop agglutinins and complement-fixing antibodies which are useful for identification purposes. Some influenza patients give positive complement fixation tests with antigens of *B. influenzæ*, but the same may be said of antigens of streptococci, and pneumococci. There are probably different strains of *B. influenzæ* as in the case of pneumococci.

WHOOPING COUGH*

Bacterium pertussis

According to latest statistics, the death-rate of whooping cough is roughly about 5.5 per 100,000 exposed. The causative agent, according to Bordet and Gengou, is an influenza-like bacillus.

It is a non-motile coccoid bacillus, stained faintly by aniline dyes and Gram-negative. It is distinguished from the influenza bacillus by agglutination and complement deviation tests and by the fact that it can be gradually adapted to ordinary media.

The production of *pertussis* in young animals has been claimed. The organism has an endotoxin which produces local necrosis after subcutaneous injection.

Further evidence on the etiology of whooping cough is afforded by the observations of Mallory and others who have found large numbers of small microörganisms corresponding morphologically with *Bact. pertussis* occurring between the cilia on the epithelial cells lining the respiratory tract in fatal cases of the disease.

HÆMORRHAGIC SEPTICÆMIA†

Bacterium bovisepticum

Hæmorrhagic septicæmia belongs to a class of similar diseases grouped under the general head of *Pasteurelloses.*

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* Prepared by Edward Fidlar.

† Prepared by M. H. Reynolds.

This disease has been reported from many portions of North America, from some sections of South America and many European countries. It is known under a variety of names, as cornstalk disease, buffalo disease, pneumo-enteritis, etc.

Bact. bovisepticum produces a serious disease and affects a wide variety of domestic and wild animals. The domestic animals most commonly affected are cattle, sheep, and goats, the disease being much more common among cattle than among other classes of stock.

The period of incubation appears to be short, six to forty-eight hours. The onset of disease is usually sudden, and the case acute. Hæmorrhagic septicæmia does not spread from herd to herd but appears in isolated outbreaks usually at wide distances apart. It is a common experience to find a serious outbreak in one herd without any appearance of the disease in another herd in an adjoining pasture, with only a barbed wire fence between. Apparently the virus exists locally and, under as yet unknown conditions of increased virulence or lowered resistance, is able to start a local outbreak. Infection is often imported with infected cattle from large stockyards.

Hæmorrhages found at autopsy constitute the most specific and characteristic clinical evidence of this disease. Its mortality is very high, running from 50 to 90 per cent.

Hæmorrhagic septicæmia of cattle, chicken cholera, and a number of other diseases belonging to this group are very similar in clinical features and the bacteria which cause these diseases are very similar in cultural and microscopic features. Yet all evidence points to the fact that *Bact. bovisepticum* acts as a specific causal agent for hæmorrhagic septicæmia of cattle.

The method of infection is still uncertain, probably occurs by both inoculation and ingestion. This disease does not appear to spread easily by simple association or ordinary contact and there is no general atmospheric distribution of *Bact. bovisepticum.*

Acute and rapidly fatal cases where the autopsy shows only trifling lesions would indicate the formation of active toxins. The characteristic hæmorrhages indicate the production of substances actively toxic for the endothelial cells of capillaries. The fact that these hæmorrhages vary in different cases from extensive subcutaneous areas to those that are scarcely visible would seem to indicate that this toxin is produced in greatly varying quantities or is of greatly varying toxicity.

The lesions produced by this bacterium indicate a general distribution through the body.

The characteristic features as previously mentioned are the hæmorrhages which are either subcutaneous, submucous or subserous. Lymph glands are frequently infiltrated with extensive hæmorrhages.

Cases have been reported as showing high fever. Those studied by the writer have, as a rule, showed slight disturbance of temperature until near death. When voluntary muscles are involved the hæmorrhages invade connective tissues rather than muscle tissue proper. Hæmorrhages of the pericardium and heart wall are especially common. The rectal and vaginal mucous membranes are often intensely hyperæmic or hæmorrhagic. Fæces, urine and nasal discharges are often blood stained.

Bact. bovisepticum resembles so closely the bacterium of chicken cholera, the bacterium of rabbit septicæmia, *Bact. suisepticus* and other members of this group (*Pasteurelloses*) that laboratory differentiation from other members of the group is exceedingly difficult. It is a very small bacterium with rounded ends, closely resembling a diplococcus. It is from 1μ to 1.5μ long and from 0.3μ to 0.6μ thick. Involution forms sometimes appear. It shows bipolar stain, decolorizes by Gram's method, produces no spores, has no flagella, and is non-motile. Short chains are not uncommon.

It grows best at body temperature and slowly at room temperature. It is killed at 58° in eight to ten minutes.

The disease resembles anthrax in some general characteristics but is easily distinguished by microscopic examination of the blood and failure to find the large anthrax bacterium and by the fact that the blood from the general circulation is apparently normal in hæmorrhagic septicæmia. This disease also resembles symptomatic anthrax (blackleg) but is easily distinguished in that external swellings are slight if present at all and do not show gas, both of these features being characteristic of blackleg. The bacillus of symptomatic anthrax may be recognized by microscopic examination as so different from *Bacterium bovisepticum* that there could be no mistaking one for the other.

Little is known concerning elimination of this bacterium from the diseased body and concerning methods of dissemination. Hence we are very much in the dark when attempting to deal with the disease produced by it.

Isolation and disinfection are to be recommended on general principles. Immunization by present methods appears to be very questionable.

LEPROSY*

Bacterium lepræ

Leprosy is a disease almost as old as history itself but modern leprosy cannot be definitely identified with the leprosy of the Old Testament, and to-day is found chiefly in oriental countries and in Norway, Iceland and Russia. The disease is present in some of the provinces of Canada and in the States of Louisiana, California and Minnesota, and practically limited to Scandinavians in the latter states. The natural incubation period is difficult to ascertain but is probably a matter of months or years.

Clinically there are two main types of the disease, the tubercular or nodular and the anæsthetic types. In the first form, nodules develop in the face or other parts of the body usually preceded by an erythematous patch. The mucous membranes become affected more or less extensively and the hair and eyebrows fall out. In the anæsthetic type after various disturbances of sensation which may sometimes be followed by maculæ there develop areas of anæsthesia. Bullæ, ulcers and necrosis may occur with resulting deformities or again this type may exist for years without leading to such results.

The bacteria of leprosy were first described by Hansen in 1879 and almost at the same time Neisser published similar descriptions. Cultivation of *Bact. lepræ* has been successful in the hands of Clegg, Duval and others.

The microörganisms can be shown in tissue by the use of the Ziehl-Nielsen or Gabbet methods.

In tissue the bacterium closely resembles the bacterium of tuberculosis, but usually appears somewhat longer (5μ to 7μ) and thicker (about 0.5μ) straighter and less beaded. Flagella have not been demonstrated. The bacterium can be stained with the ordinary aniline dyes. It is Gram-positive. The staining reactions on the whole are like those of *Bact. tuberculosis* but *Bact. lepræ* stains more readily and also decolorizes more readily; 30 per cent nitric acid followed by 95 per cent alcohol will totally decolorize them while *Bact. tuberculosis* resists. The optimum temperature for growth ranges from 32° to 35° when grown in symbiosis with amœbæ. The reaction of the media upon which successful isolation takes place is 1 to 1.5 per cent alkaline to phenolphthalein. In recently isolated cultures growth is extremely slow and appears on the surface of the special media in four to six weeks as moist grayish-white colonies elevated centrally, with an irregular wavy margin and attaining a diameter of 2 mm. Older cultures on glycerin agar are moist and abundant, and develop an orange-yellow pigment. In glycerin broth a thin membrane is formed at the surface after several weeks, while a small amount of sediment collects at the bottom of the tube leaving the medium clear. The resistance to heat is much greater than that of ordinary vegetative bacteria, so that cultures may be freed from contamination by the latter by simply heating to 60° for one hour. The resistance to drying is probably considerable.

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* Prepared by Edward Fidlar.

Human leprosy appears to be confined naturally to man and only lately has the disease been transmitted artificially to animals. In the Japanese dancing mouse, and less frequently in the white mouse and the monkey small nodules may be found on the peritoneum about four to eight weeks after intraperitoneal inoculation. The animals do not show any symptoms of illness and must be killed in order to find the lesion. More recently Duval has produced an apparently typical leprosy in monkeys by repeated injections of cultures from artificial media.

It is generally considered that the usual path of entrance of the bacterium is the naso-pharyngeal mucous membrane. The organisms seem to be distributed slowly over the body and according to their location produce the different types of the disease. They are found in the nodules of the nodular type and in the nerve trunks of the anæsthetic type.

Agglutinins have been demonstrated in the blood of lepers. Complement deviation with various antigens has been investigated and indicates an antilipoid immune body which not infrequently gives a positive Wassermann reaction. Lepers react frequently to tuberculin inoculations and this is not considered to be always due to associated tuberculosis.

The chief source of elimination of leprosy bacteria is the nasal mucosa. The bacteria have been demonstrated in this region in about 40 per cent of the macular types, 80 per cent of the nodular and mixed types.

While a great deal of popular fear exists against this disease it is decidedly less infectious than pulmonary tuberculosis. Lepers have unquestionably been subjected to a great deal of wholly unnecessary persecution.

Prophylactically, isolation has certainly demonstrated its value and the reported increase of leprosy in certain parts of Europe has been attributed to a decrease of this custom of segregation.

PLAGUE*

Bacterium pestis

Epidemics have been recognized since the second century. About half the population of the Roman Empire died in the sixth century. An epidemic of the fourteenth century destroyed half the inhabitants of Europe. In India during 1901 to 1904 about 2,000,000 died of the disease. In China, in Egypt, South Africa, and in sea ports of the Western hemisphere, plague has been found.

Among animals the disease has been found chiefly in rats and squirrels. Dogs may occasionally become infected.

Four types are described, the ambulant, bubonic, septicæmic, and pneumonic. The bubonic type forms three-quarters of the cases. Physical and mental depression accompanied by a high fever, often with a remission about the third day, occurs. Collapse may then follow with death. Glandular swellings (buboes) appear in the groin and axilla and these may suppurate. Hæmorrhages beneath skin and mucous membranes are common. The third type is a very rapid form, causing death before the development of buboes. The fourth type is also a short and extremely fatal form, marked by the occurrence of bronchopneumonia due to the plague bacteria.

The bacterium of bubonic plague was described by Yersin and Kitasato independently in 1893. They found it in glands and throughout the body in fatal cases.

The organism is readily grown from the buboes, the blood, and the sputum in the pneumonic type, by simple inoculation of ordinary media of a slightly alkaline reaction. The bacteria are 1.5μ to 1.7μ long by 0.55μ to 0.7μ wide with rounded ends occurring singly or in pairs and short chains in exudates and sometimes in long chains in broth. Involution forms, large swollen spheres, clubs, etc., are characteristic in artificial media. There are no spores. It is non-motile. Some observers have demonstrated a gelatinous capsule. Occasionally very distinct branching occurs (Hill). It stains easily with aniline dyes, particularly at the poles which may show round or oval granules. It is Gram-negative. Its minimum temperature for growth is about 12°, the optimum 30°, the maximum 40°. It is aerobic. Agar after twenty-four hours shows small granular grayish colonies with a thickened center and indented margin. Broth shows a granular deposit and sometimes a pellicle with dependent outgrowths, the medium remaining otherwise clear.. Gelatin growths are as on agar, and the medium is not liquefied. Litmus milk may show slight acid formation and no coagulation. Potato shows nothing characteristic. The toxins appear to be largely endotoxins, though soluble poisons have been found in old cultures. No indol is formed. Resistance toward heat is not great, boiling kills in a few minutes. Light kills in a few hours. They do not resist drying well, but in a moist condition remain viable for over a year. The usual strengths of ordinary disinfectants kill in about ten minutes.

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* Prepared by Edward Fidlar.

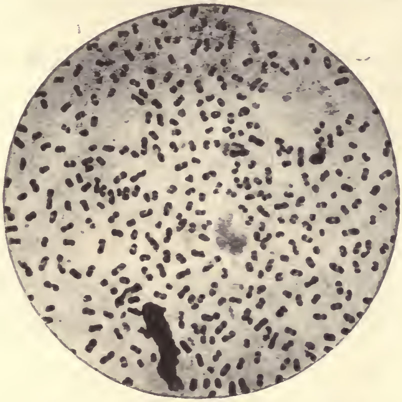

FIG. 173.—*Bact. pestis.* (*After Yersin from Williams.*)

Rats, mice, guinea-pigs, rabbits, and monkeys are particularly susceptible to inoculation and even insects die from infection.

The bacterium enters the body through the (usually abraded) skin or respiratory tract. After involvement of the nearest lymphatic glands the bacteria are distributed through the blood.

Single attacks immunize. The antibodies developed are agglutinins, probably bacteriolysins, and possibly antitoxins. The agglutination reaction is of value in diagnosis.

The organisms are eliminated in the exudates from suppurating buboes, in the sputum in the pneumonic type, and are present throughout the body after death. The dead bodies of human beings and of rats are sources of infection for other rats. There seems good evidence of these animals becoming chronic carriers though showing no symptoms of disease, and may thus be important factors in maintaining and spreading plague.

The disease is largely communicated by means of fleas which have become infected by living on other human beings or even upon rats.

Prophylaxis consists of isolation of pneumonic cases, thorough disinfection involving the killing of fleas, and chiefly the destruction of rats, squirrels, and other animals which may serve as carriers. Haffkine's vaccination method has also been shown to be a valuable prophylactic measure.

The serum of immunized animals has been tried as a therapeutic agent and gives encouraging results when administered in the early stages.

SWINE ERYSIPELAS*

Bacterium rhusiopathiæ suis

Swine erysipelas is an infectious disease of hogs characterized by red or violet discoloration of the skin and mucous membranes. Swine erysipelas does not exist in the United States but is very prevalent in continental Europe. It is caused by a very small, slender, non-motile, non-spore-bearing bacterium (*Bact. rhusiopathiæ suis*) which stains by Gram's method, and grows feebly on the ordinary culture media. Development is best under anaerobic conditions. In gelatin stab cultures, after three or four days, a white growth can be seen along the needle puncture. Radiating from this are a number of delicate tufts which give the growth the appearance of a fine test-tube brush. White and gray mice, white rats, and pigeons succumb to the inoculation of minute amounts of the culture. The bacteria tend to collect within the bodies of the leucocytes. This microörganism is closely related to and possibly identical with the bacterium of mouse septicæmia (*Bact. murisepticum*). Preventive inoculation with attenuated cultures has long been practiced successfully in Europe.

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* Prepared by M. Dorset.

TUBERCULOSIS*

Bacterium tuberculosis

Consumption, phthisis, scrofula, pearl disease, etc., are synonyms of the term tuberculosis.

This bacterium in its several varieties produces a very universal disease; practically all common animals and man are subject to it. Cattle and swine among the domestic animals are especially susceptible to this infection and wild animals in captivity easily become affected.

The normal progress of tuberculosis is slow. Its characteristic feature is the tubercle or nodule of various sizes.

Tuberculosis is probably the most common and serious of all diseases for either animal or man.

In 1918, 150,000 persons died from tuberculosis in the United States, or at the rate of 150 per 100,000 population. Based upon these facts, it is estimated that about 10,000,000 of those now living in the United States may die of the disease. It is claimed that the disease alone costs the United States from $400,000,000 to $1,000,000,000 each year (Fisher).

If the loss from wage earnings, the cost of the patient in suffering, medical treatment, medicines, nursing, board, and care, also the suffering and sacrifice entailed by near relatives, friends, and communities are considered, the loss to the country mentioned above does not appear so enormous.

It is estimated by the United States Bureau of Animal Industry that 2 per cent of hogs in the United States are tubercular, and that losses of stock in the United States, due to tuberculosis, amount to $23,000,000 annually. Of 400,000 cattle tested in many states of the United States during a certain period 9.25 per cent were tubercular. The highest prevalence of tuberculosis in cattle is among pure bred herds and in city dairy stables; *i.e.*, among the cattle kept most closely confined. It is most common in old cattle and rare in calves under six months old. Tuberculous infection is quite generally scattered among cattle of civilized nations.

Tuberculosis appears in man usually in the form of lupus (tuberculosis of the skin), or scrofula (tuberculosis of the cervical glands), or phthisis (tuberculosis of the lungs). It also frequently appears in the mesenteric glands and other glands of the body, and may appear in any of the tissues. It is quite possible, judging from autopsies, that many persons have tuberculosis without realizing its existence in the body, and without its being detected in any way. It is questionable, however, whether under such circumstances the disease is transmitted or disseminated.

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* Prepared by M. H. Reynolds.

As a rule affected cattle show no definite outward signs of the disease. Badly diseased animals sometimes appear poor and unthrifty. Many cases are mild and latent. A few tubercular animals cough; some show harsh hair and skin and other expressions of ill health. While these symptoms do not necessarily indicate tuberculosis, they are very suggestive.

Bact. tuberculosis may invade almost any tissue or organ of man or the animal body and produce a variety of lesions. Man usually gives some evidence of the disease either objectively or subjectively, and in many instances the disease assumes a definite form which is easily recognized by medical men, unlike its presence in animals. The symptoms are more evident in swine than in cattle. Affected hogs are often unthrifty and show glandular enlargements and degenerations of the enlarged glands.

Avian tubercle bacteria are becoming disseminated among poultry, and to a serious extent in some sections of the country. Among the more prominent symptoms of avian tuberculosis are emaciation with marked anæmia and weakness. Examination of the carcass shows disease most frequently in the liver, but intestines, spleen, lungs, and even the skin may be invaded. Danish authorities report* serious outbreaks among swine due to avian tubercle bacteria.

It has long been firmly established that *Bact. tuberculosis* is the specific cause of this disease. But while this bacterium is to be regarded as the specific cause it must be understood that this organism is frequently associated with pus-producing bacteria which are responsible for certain phases of the disease as commonly seen. It should be understood also that persons and animals become more susceptible and have greater opportunities for infection under close confinement and lack of exercise. There has been great difference of opinion concerning the unity of the tubercle bacterium, and the probability of inter-transmission between man and the lower animals. A large number of bacteriologists now hold that the several types of tubercle bacteria are but environmental variations of the same species. In any case, man clearly appears susceptible to both human and bovine types at least.

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* Dunne, Trans. Jour. Bd. Agr. (London), 22 (1915), No. 1.

The entrance of the germ may occur in four ways, namely, by way of the digestive tract; it may occur by way of the respiratory organs; it may occur by inoculation; and infection may possibly occur before birth. Some authorities hold that the most common infection is by way of the digestive tract and in early life. Others hold that inhalation tuberculosis is most common.

This bacterium produces a slow toxæmia, and it is this toxæmia together with physical embarrassment of the vital organs by extensive lesions which together harm the affected body. Toxic substances are produced, as indicated by the fact that killed cultures by subcutaneous injection may destroy local tissues and produce abscess, debility, and emaciation. Production of toxins is indicated by the further fact that certain antitoxic immunity may be produced by minute doses of killed culture gradually increased.

Tuberculin is a common and well known product or mixture of products of this bacterium. One of its constituent products has been reported as a fever producer. Another product has been reported which reduces temperature, and still another which produces convulsions, in sufficient dose.

Tuberculosis may be very general. Almost any tissue or organ in the body may be invaded; but as a rule, not many organs are badly affected in the same case. Distribution occurs by way of both the blood and lymph streams, especially the latter. It seems probable that tubercle bacteria may be distributed in the body by wandering phagocytes.

The *Bact. tuberculosis* has a characteristic tendency to produce tubercles or nodules which may be large or small and which have a tendency to central necrosis and degeneration. Mucous membranes, under this infection, tend to develop superficial ulcers.

The lesions produced by this microörganism may vary from the tiniest tubercles to extensive areas of large organs. Lymph glands frequently enlarge and undergo cheesy or calcareous degeneration. Tubercular masses of various sizes may appear upon the lining membranes of the chest and abdominal cavities and upon various internal organs. Cheesy abscesses may appear in the depths of soft organs. In cows the udder is occasionally enlarged and shows hard masses with little or none of the heat usually occurring in connection with inflammatory changes. Bones and joints are often involved especially in the human; these structures increase in size, produce pain, and suppurate.

Bacterium tuberculosis is a slender rod-shaped organism with rounded ends and under certain conditions shows granular forms. It varies between 2μ and 5μ in length, and 0.3μ to 0.5μ in width. This bacterium is usually straight, but may be bent; it appears either singly or in groups or branched, non-motile, and is probably not a spore producer (Fig. 174). Glycerin agar, blood serum, egg slant, and bouillon may all serve as satisfactory nutrient media. It is aerobic and its temperature limits for growth appear to be 29°-42°C. With the exception of young and rapidly growing forms it is strongly acid-fast. Tubercle bacteria may be demonstrated in cover-glass smears from diseased tissues and fluids and in tissue sections (Fig. 175). In human tuberculosis the bacteria are frequently determined in the sputum, in bovine tuberculosis the bacteria may be occasionally demonstrated in the nasal discharges and in the manure. Positive diagnosis may usually be made by guinea-pig inoculation. For microscopic examination a cover-glass smear is fixed in the usual way, then stained with hot carbol-fuchsin three to five minutes or in cold stain fifteen to twenty minutes. It is then decolorized, *e.g.*, in 10 per cent nitric acid, and counterstained with methylene blue for about one minute, after which it is rinsed and ready for examination.

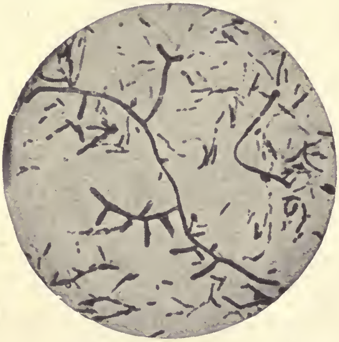

FIG. 174.—*Bact. tuberculosis.* Branching forms from a culture. (*After Migula.*)

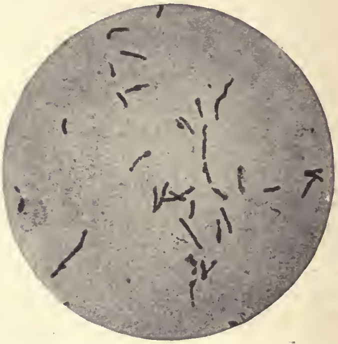

FIG. 175.—*Bacterium tuberculosis.* Sputum preparation uncolored. (*After Migula.*)

It is conceded that tubercle bacteria do not multiply in nature outside the animal body and, therefore, dissemination must depend wholly upon the dissemination of infected people or animals and materials infected by diseased men and animals. Tubercle bacteria escape from open ulcers or from tubercular lesions which connect with digestive or respiratory organs. They may reach the surface in other ways; *e.g.*, by the discharge of abscesses.

In controlling tuberculosis among humans at the present time, several methods are in vogue. In some localities, an effort is made to segregate tuberculous patients during the day for the purpose of treating them as well as teaching them how to care for themselves. This method aims to instruct how to prevent dissemination and transmission of the disease, to prepare suitable nourishment, and to secure the advantages of open-air influences. This instruction not only extends to the patients but others with whom the patients may mingle. Sanitaria are also constructed to receive patients suffering from the disease, and care for them under suitable medical supervision by proper treatment, nourishment, and open-air life. Again, the policy is being inaugurated to instruct tuberculous patients, where it is impossible to reach them by other means, to care for themselves in their own homes.

By these general hygienic measures, much good has been accomplished, not only for the patients but, also, in a diminution of the number of new cases developing.

The animal disease is carried to distant points, most commonly by breeding stock. Locally the disease spreads either by the movement of affected cattle, or frequently

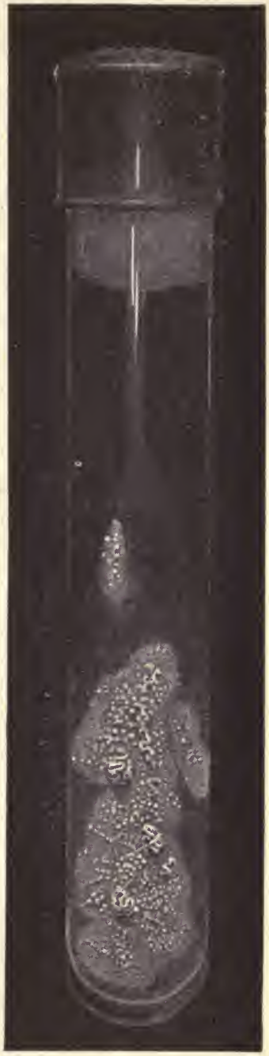

FIG. 176.—*Bact. tuberculosis.* Glycerin agar culture (*After Curtis from Stitt.*)

by infected milk. Hogs receive their infection from the milk of tubercular cattle or from the manure or carcasses of such cattle in feeding yards. Unventilated stables are favorable for the spread of this disease because with insufficient ventilation the bacteria are not carried out, but become constantly more numerous. The tubercle bacterium is quite resistant to drying, but is rather sensitive to sunlight. It is usually destroyed by moist heat in six hours at 55°; in twenty minutes at 60°; and generally in five to twenty minutes at 95°, depending upon the protection it may have.

Conditions of sensible sanitation are of the utmost importance. These include exercise, sunlight, and ventilation, particularly sunlight. In order that effective control work may be done among animals, tuberculin must be used freely and conscientiously.

The method of dealing with diseased herds depends upon breeding and value. Common cattle are usually dealt with most economically and efficiently by slaughter with a view to using such carcasses as may pass inspection. Valuable cattle, especially pure bred animals, may be used for breeding purposes, gradually building up a sound herd and gradually displacing the diseased animals. This latter plan is usually unprofitable and unwise except for very valuable cattle.

FOOT ROT OF SHEEP*

Bacillus necrophorus

This is an infectious disease of sheep characterized by an ulcerative inflammation of the tissues just above the horny part of the cleft of the hoof. It is seen in Europe, England, Australia, and the United States. Sheep are made lame and if the disease is not checked by appropriate treatment, the hoof becomes greatly distorted, the sheep being finally unable to walk. Mohler and Washburn† state that foot rot is caused by *B. necrophorus*, this organism being associated with pus-producing micrococci. *B. necrophorus*, which is a strict anaerobe, tends to grow out into long filaments; it is stained by the ordinary aniline dyes, but not by Gram's method. Rabbits and white mice are susceptible to inoculations of this bacillus, but guinea-pigs appear to be immune. The disease is treated by causing infected sheep to walk through a disinfecting solution, such as a 3 per cent solution of carbolic acid.

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* Prepared by M. Dorset.

† Bull. 63. Bur. An. Industry, U. S. Dept. Agr., 1904.

MALIGNANT ŒDEMA*

Bacillus œdematis maligni

The disease occurs as the result of infection of wounds with dust or soil. The wounds must involve the tissues deeply as in compound fractures and deep cuts.

Any animal may be infected, although the dog and cat are said to be rather more resistant than others. The guinea pig is very susceptible.

The incubation period is short, from one to two days as a rule.

The usual case begins with sudden spreading hæmorrhagic, subcutaneous œdema and high fever. Practically no gas is formed. The fluid shows bacilli both with and without spores. Where soil contamination exists, mixed infections with gangrene are common.

Pasteur in 1877 and Koch and Gaffky in 1881 found and studied the organism and by passing from animal to animal established the causal relationship.

Glucose agar or glucose gelatin is inoculated with the suspected fluid, plates poured and placed under anaerobic conditions. The organism is 0.8μ to 1μ wide. Filaments may occur. The rods without spores are uniform in width with slightly squared ends. They are usually single, though pairs end to end are frequent and chains are also found. Oval spores are formed somewhat variable in their position, with a diameter usually larger than that of the vegetative rod, bringing about a spindle shape. Peritrichic flagella have been demonstrated, about twenty in number. It stains readily with aniline dyes, usually Gram-negative though somewhat variable and indefinite in this regard. Growth takes place at both 20° and 37°. It is a strict anaerobe. Like anaerobes in general it prefers the presence of a fermentable carbohydrate such as glucose. On agar the colonies are small, whitish, and irregular in outline. Gelatin and blood serum are digested, caseinogen is changed to casein which is then digested. In both protein and carbohydrate media a gas is produced which has a very disagreeable odor. The spores are very resistant.

This resistance accounts for its continuous presence in earth and dust and as a constant inhabitant of the intestine of animals, especially of herbivora.

SYMPTOMATIC ANTHRAX OR BLACKLEG†

Bacillus anthracis symptomatici (*Bacillus chauvæi*)

Blackleg, black quarter, symptomatic anthrax, quarter ill, are synonyms employed to designate this disease.

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* Prepared by Edward Fidlar.

† Prepared by M. H. Reynolds.

Symptomatic anthrax is a very old disease and until recent years has been confused with true anthrax. This disease is widely distributed, affecting practically all countries and climates.

It is enzoötic, never spreading widely or rapidly, and is often found in certain infected valleys and in relatively small areas. Young cattle, generally under two years of age, are most commonly affected, but sheep and goats are susceptible to this infection.

This disease is infectious by inoculation, perhaps also by ingestion, and usually acute. Subcutaneous and muscular tissues are especially affected. Its most prominent and characteristic feature is swelling, affecting most frequently, the front or hind quarters, and not extending below the knee or hock. As a rule, the bacillus of symptomatic anthrax produces a very acute disease with severe constitutional disturbances, and early death.

The bacillus of symptomatic anthrax has been clearly demonstrated to be the specific cause of blackleg. The period of incubation in the natural disease is uncertain. Under artificial inoculation this period varies from two to three days and is occasionally as short as one day.

This bacillus produces in culture a very active toxin. This toxin is quite resistant to heat. That the bacillus of symptomatic anthrax stimulates the production of antibodies and that the injury is done by toxins, is shown by the fact that immunity against virulent culture may be produced by treatment with presumably sterile filtrates of virulent cultures.

The bacillus of symptomatic anthrax is rarely found in the general blood before death; but is abundant in the affected muscle and overlying subcutaneous tissue. It also occurs in great numbers in the bile and intestinal contents.

Mucous membranes become congested and then very dark. There is a high fever. Local swellings occur which are at first sensitive and later insensitive and gaseous. There is usually developed a very marked swelling of a front or hind quarter or of the neck, with rapid formation of gas. The serous membranes, particularly the pleura and peritoneum, develop severe inflammation with hæmorrhages and infiltrations and corresponding exudation in the cavities. General decomposition is rapid and the swelling may show a slight acetone odor. The local subcutaneous tissues are infiltrated, hæmorrhagic or gaseous. The local lymph glands are swollen and hæmorrhagic or œdematous. Muscle fibers show various degenerative changes. The abundant gases are mostly hydrogen and carbon dioxide. Blood from the general circulation is normal as to color and coagulation.

B. anthracis symptomatici is about 3μ to 6μ long by 0.5μ to 0.8μ thick. This is a spore-bearing bacillus of drum-stick shape or spindle shape and is anaerobic. It grows best at about 37°. It stains either by the simple aniline dyes or by Gram's method. In artificial cultures, it sometimes shows long forms. This organism is motile for a short time, but soon loses this power, probably on account of the oxygen to which it is exposed. It shows well-defined flagella and develops spores. The specific organism may be demonstrated by the microscope in the blood without staining if done soon after death.

The bacillus of symptomatic anthrax is easily demonstrated in cover-glass smears from the affected tissues, and is very different from the bacteria of anthrax and hæmorrhagic septicæmia, the only diseases liable to be mistaken for blackleg excepting malignant œdema. Anthrax is aerobic. Symptomatic anthrax is anaerobic. This organism may also be demonstrated by animal inoculation. The guinea-pig serves well for this purpose; it is very susceptible to inoculation and gives a characteristic blackleg reaction in both symptoms and lesions. From the lesions thus produced the characteristic bacilli are easily demonstrated by the microscope.

Elimination of this virus from the body occurs chiefly in the various discharges, and especially in the manure and also in general decomposition of the carcass. Dissemination of this disease is chiefly if not exclusively by diseased carcasses and parts of carcasses and by the discharges. Contaminated soil plays a very important part in the prevalence of this disease. It appears possible that the specific bacillus may even multiply in the soil.

Carcasses should be burned if possible, otherwise very deeply buried and covered with lime. Contaminated grounds, or stable floors must be thoroughly disinfected, for the infection is very persistent and difficult to eradicate except by most vigorous effort since the spores are very resistant to heat and drying. Preventive inoculation after the method of Arloing as improved by Kitt is very satisfactory. Their vaccine consists of specially treated muscular tissues from the diseased part.

TETANUS*

Bacillus tetani

This disease is found throughout the world but more frequently in warmer than in colder climates. Certain localities are particularly affected. Man and domestic animals are susceptible.

The incubation period varies: a few hours in the case of small animals receiving injections of toxin; several days or weeks in cases of natural infection in man, or even several months as in some cases of wounded soldiers who had received injections of protective serum. Tetanus has followed operations in which old healed wounds have been opened up.

Under natural conditions the disease follows a wound of a punctured type with contamination by earth, especially in injuries of hands and feet.

It is characterized by tonic spasms of the voluntary musculature usually beginning in some one group of muscles and finally becoming general. The parts first affected are, in cases artificially produced, those at the site of inoculation, but in natural infections in man it is more common for the disease to manifest itself by stiffening of the muscles of the neck and face, producing what is ordinarily termed "lockjaw." In less severe infections in man local pain and stiffness are the first indications. The spasms occur in paroxysms which are spontaneous or excited by effort. They are more or less prolonged and exhausting and are accompanied by greater or less pain. Death results from general loss of strength or involvement of the respiratory muscles. The shorter the incubation period the higher the mortality. Few recover when the incubation period is less than ten days, about half the cases recover when the period is more than fifteen days. In the British army in the first two years of the war, the mortality ran over 50 per cent, and after that about 25 per cent.

The nerves may show injury as indicated by swelling and redness and microscopically nerve cells have been observed in a state of granular degeneration; there is a more or less distinct general congestion of the organs.

While lockjaw has been known clinically for centuries, it was not until 1884 that the infectious character was demonstrated when Carlo and Rattone and Nicolaier were successful in animal inoculations. Kitasato obtained pure cultures of the bacillus in 1889.

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* Prepared by Edward Fidlar.

The organisms may be detected rarely by examination of stained preparations of the pus from the wound. Pure cultures may be obtained by inoculating an alkaline dextrose broth with pus or tissue, incubating under anaerobic conditions for about forty-eight hours until sporulation, then exposing half an hour to a temperature of 80° to kill all vegetative forms and subsequently making subcultures. If other spore-bearing bacteria are present considerable difficulty may be encountered. Subcutaneous inoculations of mice or guinea-pigs is a good method for demonstrating the presence of the organism, but pure cultures should be combined with some aerobe (say *B. coli*) to secure results.

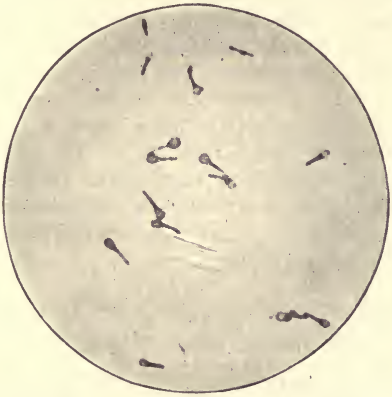

FIG. 177.—Tetanus bacilli showing end spores. (*After Kolle and Wassermann from Still.*)

The *B. tetani* is about 2μ to 5μ in length by 0.3μ to 0.8μ in width with rounded ends. The vegetative rods are uniformly cylindrical but the terminal spores give a "drum stick" appearance (Fig. 177). The arrangement is usually single, but threads may occur especially in old cultures. The organism forms round terminal spores which have a diameter of 1μ to 1.5μ. The young bacilli are motile and possess 50 to 70 peritrichic flagella. Motility is lost with sporulation. The bacillus is stained by the aniline dyes and is Gram-positive. The spores are readily demonstrated by the special stains. The range of temperature for growth is from about 14° to 45° with an optimum about 37°. The organism is usually considered an obligate anaerobe though experimentally aerobic strains have been developed but with loss of pathogenic and toxogenic properties. Pure cultures do not develop in an atmosphere of carbon dioxide. Media for the cultivation of the bacillus should be slightly alkaline and should contain for best growth about 2 per cent of glucose or 1.5 per cent sodium formate. The addition of pieces of fresh raw sterile tissue is valuable. On agar at 37° colonies appear in forty-eight hours which show microscopically, a mass of tangled threads resembling colonies of *B. subtilis* or *Bact. anthracis.* In broth a cloudiness is produced in twenty-four to thirty-six hours with the development of gas and a very disagreeable odor. In gelatin the colonies develop more slowly than on agar and show liquefaction. In old stab cultures a pine tree growth occurs. Gas is usually produced. In milk growth occurs without coagulation. Acid is produced in some carbohydrate media. Gas is produced during the action upon protein and consists chiefly of carbon dioxide but also of hydrogen sulphide and certain volatile organic compounds commonly found in putrefactions. The tetanus bacillus forms two soluble toxins, tetanolysin, and tetano-spasmin. The former is less stable and dissolves red blood-corpuscles. The latter produces the characteristic spasms of the muscles. This poison may be obtained after one to two weeks' growth in slightly alkaline salt-peptone-bouillon under anaerobic conditions at 37.5° and separated by filtration through porcelain filters. When taken by the mouth the toxin is ineffective, given intravenously it produces a generalized tetanus, while after subcutaneous injection the disease begins with local spasms. The central nervous system is reached by ascent of the toxin along the motor nerves nearest the point of inoculation. A dose of toxin injected directly into the nerve trunk of an animal may produce a fatal result when it is innocuous intravenously. The spores often withstand 80° for one hour and live steam for about ten minutes. Direct sunlight destroys them in time. They survive drying for several years and resist the ordinary disinfectants for a considerable length of time, 1:1000 mercuric chloride for three hours, 5 per cent carbolic acid for about ten hours.

Practically all mammalia are susceptible to tetanus though rats are but slightly so. Very minute doses of toxin suffice to kill mice and guinea-pigs. Birds show but little susceptibility and the hen is said to be three hundred thousand times more resistant to tetanus toxin than the horse. Reptiles and amphibians are practically immune to very large doses when kept at low temperature.

Natural infections probably do not occur without the presence of other microörganisms. The bacillus and its associated material gains entrance through some break in the tissues. The organism is practically confined to the site of inoculation, but it is sometimes found in the blood and internal organs after death.

Against toxin-freed cultures phagocytosis is probably the process which overcomes infection. The toxin is highly antigenic and animals can be immunized against it in a manner similar to that for diphtheria toxin.

While direct infection of one person from another has occurred, cases of human tetanus are very rarely responsible for others.

Horses and cattle are chiefly responsible for its distribution, the tetanus bacillus being common in manure, which accounts for the occurrence of tetanus in soil-contaminated injuries.

Tetanus antitoxin, as a prophylactic measure, is widely and successfully used in all suspicious wounds in civil life, and was extremely valuable during the war. Its administration should be combined during the first twelve hours after injury, with thorough surgical treatment aimed at the removal of damaged tissue and foreign bodies. The passive immunity begins to decline in about ten days. The serum is less effective as a therapeutic measure, and must be given as soon as possible after the detection of symptoms in very large doses, intrathecally, intravenously and subcutaneously.

TYPHOID FEVER*

Bacillus typhosus

Typhoid fever is one of the most widespread of bacterial diseases and is found endemic in practically all the countries of the world. Epidemics frequently occur because of the infection of some local public utility related to food or drink, particularly water or milk.

Typhoid fever occurs naturally only in man. Intraperitoneal inoculation of susceptible animals may result in death with acute peritonitis, but lesions are in no way specific and can be produced by the colon bacillus.

The period of incubation varies ordinarily from five to twenty-one days, with an average of fourteen days.

The first week of the disease in man begins with a train of rather indefinite symptoms such as headache, loss of appetite, digestive disturbances, lassitude, and sleeplessness. Nose bleed is a peculiar and rather constant feature. The temperature and pulse gradually rise until by the end of five to seven days the former has become high, 103°F. to 104°F. and constant. The temperature continues thus through the second week during which a gradual stupor and occasional delirium, diarrhœa, and enlargement of the spleen occur. The pulse is often dicrotic and there is a rash consisting of isolated flattened rose-colored macules or spots which may be few or numerous and occur in successive crops. During the third week in mild cases these symptoms gradually subside. In severer forms no abatement is shown and complications are liable to occur. The fourth week shows beginning convalescence in the typical case.

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* Prepared by Edward Fidlar.

The characteristic pathological findings are swelling and ulceration of the lymphoid structures of the lower part of the small intestine best seen in the Peyer's patches of the ileum just above the ileo-cecal valve. The mesenteric glands and spleen are hyperæmic. Parenchymatous degenerations more or less severe may be found in other organs. The characteristic histological feature is the crowding of the lymph spaces by proliferated endothelial cells.

Perforation and hemorrhage of the bowel, peritonitis, myocarditis, thrombosis, etc., render typhoid fever a dangerous disease. The fatality varies considerably; at one time estimated at 25 per cent, it has been brought down to 10 to 15 per cent by modern methods of treatment and has been given in Minnesota as low as 4 per cent.

Eberth found the organism in 1880 by the examination of the mesenteric glands and spleen of fatal cases. Gaffky cultivated it in 1884. The causal relationship has been a matter of gradual acceptance through evidence furnished by the study of such immunity processes as agglutination, bacteriolysis, and complement deviation, and finally by the high percentage of positive blood cultures. Conclusive evidence is afforded by the development of typhoid fever following the ingestion of pure cultures with suicidal intent.

The agglutination reaction of Gruber and Widal is universally employed in diagnostic laboratories. The blood serum of typhoid patients, after a certain period of the disease, will cause a characteristic clumping of the bacilli when mixed with pure cultures. The fresh serum from a clot may be used, or, more conveniently, dried blood from which a watery extract can be made. In positive cases the reaction is present in at least the one-fiftieth dilution and usually in the one-hundredth or higher dilutions. The culture employed should be eighteen to twenty-four hours old; it should be freely agglutinable and show no artificial clumping, characters not possessed by all strains, especially those recently isolated. Cultures killed by a small percentage of carbolic acid have been recommended for constancy in place of the living organisms. When the microscopic method is used the reaction should be distinct in about one hour.

Owing to the extensive adoption of inoculation against typhoid and paratyphoid fevers, the problem of exact diagnosis has become more difficult. If culture fails to reveal the exciting organism it becomes necessary to take a series of at least three agglutination tests at intervals of two or three days in order to determine the comparative curves of agglutinin for *B. typhosus*, *B. paratyphosus A.* and *B. paratyphosus B.* For this purpose Dreyer's technique has been largely used.

An immune body capable of binding complement in the presence of typhoid antigen is said to occur in typhoid sera before the agglutinative property appears.

The detection of the typhoid bacillus in the circulating blood has been very widely successful and furnishes the best support for the diagnosis of the disease. While blood culture may be hardly practical in public health laboratories, it has become a routine measure in the modern hospital. Blood is taken aseptically from a vein and about 1 to 5 c.c. is introduced into culture media, of which fluid media containing ox-bile and agar plating media containing glucose have been most strongly recommended. The fluid media are used in 100 to 500 c.c. amounts, which serves to dilute the antibacterial properties of the blood while the bile acts as an anticoagulant and possibly also as an antibactericidal measure. Plating lessens the diffusion of the antibacterial properties and thus favors growth.

The urine and fæces have sometimes to be examined for the presence of *B. typhosus*. It then becomes necessary to differentiate the colonies of this bacillus from those of the colon group. For this purpose many special media have been devised, some depending on the motility of the typhoid bacillus to form a different shaped colony in suitable soft media, others based on the fact that some substances such as fuchsin, crystal violet, malachite green, etc., inhibit the growth of associated organisms while permitting the typhoid bacillus to develop more or less luxuriantly.

As found in pure cultures, the bacillus is about 1μ to 3.5μ in length and 0.5μ to 0.8μ in width (Fig. 178). Filaments are sometimes found several times the length of the single organism. It is quite regular in shape, straight with rounded ends. The bacilli usually occur singly; occasionally two may be attached end to end for a short time. There are ten to fourteen comparatively stout flagella about two or three times the length of the organism peritrichic in their arrangement. There are no capsules and no spores. They stain with all aniline dyes, and not infrequently exhibit more deeply staining areas at the poles. They are Gram-negative. Biological and biochemical characters:—The minimum temperature is about 10°, the optimum 37°, maximum 40 to 41°. It is aerobic and facultatively anaerobic. The slight preference for oxygen is probably of little account when such sugars as glucose are present. The bacillus is not very sensitive to the reaction of media and will grow in the presence of either slightly alkaline or acid reaction. Alkaline substances are produced from peptone. Acid is formed from dextrose, levulose, galactose, mannit, maltose, and dextrin. Lactose and saccharose remain unchanged. Gas is never formed. It is the rule that the *Bacillus typhosus* does not form indol; certain strains, however, form a trace. The toxins of the bacillus have been very widely studied and several different opinions are held with regard to their nature. Most evidence supports the idea that the poisons are only set free by the destruction of the bacterial bodies. This may be brought about experimentally by various means such as the use of lytic or bactericidal sera, by the disintegration occurring in old cultures, by extraction under great pressures, by sriturating after freezing in liquid air and by emulsifying cultures, sterilizing by heat, then extracting with salt solution. These endotoxins, however obtained outtide of the host, have been found to produce by injection into animals only lytic and bactericidal sera and not an antitoxin. More recently, however, some observers claim to have shown in comparatively young cultures the presence of a substance which upon injection into animals yields an antitoxin and thus comports itself

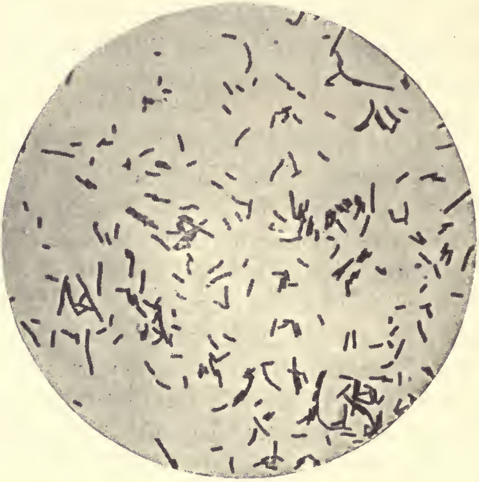

FIG. 178.—Bacillus of typhoid fever. × 1000. (*After Williams.*)

after the manner of a true diffusible or soluble toxin. Agar streak cultures show an abundant filiform whitish or bluish-gray translucent growth with no special characteristics. Broth is uniformly and moderately clouded and only occasionally a delicate pellicle may develop. Gelatin colonies are bluish white in color, transparent and with somewhat notched margins. Stab cultures show more growth at the surface, while in the depth the growth is filiform and less abundant. The medium is not liquefied. Milk is not coagulated. In litmus milk there may be a trace of acid formed at first, followed by a return to neutral or very slightly alkaline reaction. Potato was at one time considered a very valuable differential medium. The growth of the bacillus upon it is quite abundant, glistening, but invisible, when the potato is acid. A more alkaline reaction allows a rather heavy yellowish growth indistinguishable from *B. coli*. Special media are used in the cultivation of the typhoid bacillus, chiefly for differential purposes. The cultural features on these do not show sufficiently striking characters to make it worth while to review the many that have been devised. Specific agglutinating and bacteriolytic sera as well as the complement binding reaction are valuable aids in identifying the bacillus. Resistance to heat and light is not different from that of the average non-spore-bearing species. Its thermal death-point is about 56° for ten minutes, 60° for one minute. Exceptionally resistant forms have been found alive in ice after three months. Sometimes the bacilli will remain viable for a month after drying. At other times they die out rapidly. They have been found to be viable for ten days in distilled water, while pure sodium chloride dissolved exerted an unfavorable influence. In fæces the length of life is from a few hours to several days, or even as high as five months in winter. Their life in privies and cesspools is ordinarily brief but has been found to extend for thirty days. Of the non-spore-formers the bacillus appears to be rather more resistant than the average but succumbs within five minutes to 1 : 5000 mercuric chloride or 5 per cent phenol.

The organism enters the body through the mouth by means of infected fingers, food, milk, and water, etc.

On reaching the intestine the organism probably propagates to some extent before penetrating the intestinal mucosa. It enters into the blood stream and is disseminated throughout the body. According to the endotoxin theory it must slowly be dissolved by the lytic substances which have been gradually accumulating in response to the primary intoxication.

The organisms have been cultivated from the rose spots and have been found in vomit without the presence of blood, and in sputum. Typhoid meningitis and osteitis occur occasionally. At autopsy the spleen and gall-bladder yield the highest number of positive cultures. It is of interest to note too that while the highest percentage (89–90 per cent) of positive blood cultures occurs in the first week and the percentage diminishes from then on, the number of positive findings in the fæces, on the other hand, runs in the opposite direction.

Generally speaking, one attack confers immunity. Upon what antibodies immunity and recovery depend is a matter of controversy.

The elimination of the bacilli from the body will largely depend upon the stage of the disease, since the blood, especially early in the illness, practically always contains the specific organism; epistaxis is not an unimportant feature as a possible means of disseminating the germs. The bacilli can also escape in the fæces, urine, sputum, and vomit.

In the control of this disease the best place to begin is at the bedside. Disinfection of all excreta and of everything which comes into contact with the patient should be rigorously carried out and in the case of the fæces and urine should ideally be continued until examination can be made showing absence of the organism. It has been estimated that as high as 5 per cent of convalescents continue to excrete living typhoid bacilli for varying periods from months to years after the disease; the longest time noted has been forty-six years.

The recognition of typhoid carriers will depend absolutely on the finding of the specific germ in the fæces or urine as the case may be. Where there are large numbers of suspects, the opsonic index is claimed to be an aid in exclusion of the improbable ones, as well as the agglutinin reaction.

In a general way, prompt recognition of the source of infection such as milk, polluted water, bacilli-carriers, etc., together with instruction of the individual and the public are often effective in limiting and ending an epidemic.

While a great many sera have been used therapeutically with some success, prophylaxis promises more where it can be widely employed as in armies and navies. The artificial immunity is brought about by injection of dead cultures. A difference of about 25 per cent has been noted between the percentage of cases in vaccinated and unvaccinated persons in civil life.

In the United States Army, the establishment of compulsory antityphoid inoculation demonstrated most remarkably favorable results during the year 1913. *Amongst* 90,646 *men, both American and native troops, only three cases of typhoid fever occurred and two of these were infected before enlistment; there were no deaths. When comparison was made with the best results obtained in the army from sanitary measures* *alone without the vaccination, Major Russell estimated that in* 1913 *there was "only one one-hundred-and-sixty-seventh of the loss of time from duty because of typhoid fever."*

Antityphoid inoculation was of inestimable value in the great war. Exact figures cannot be given, but as an indication of what might have occurred without it, the fact has been mentioned that a small portion of the French army which in the early critical days had to be hurried to the front without inoculation, developed as many cases of typhoid fever as had occurred in the British army during the whole Boer war.

Asiatic Cholera*

Microspira comma

The disease is endemic in parts of India whence epidemics have spread throughout the world. America has been visited by several epidemics and at the sea ports more frequently, chiefly New Orleans.

The disease occurs naturally only in man. The incubation period is from part of a day to ten days, usually about three days.

In its most characteristic form the disease begins with few or no prodromata. It is marked by fever, sudden onset of purging and vomiting followed by cramps and severe depression. Evacuations finally become almost a colorless liquid, "rice-water stools." The cramps may occur in the whole muscular system most frequently in the legs and are often extremely painful. A stage of complete collapse finally occurs. There are, however, many variations from these typical cases. The mortality is usually given at from 45 to 50 per cent.

After death there are found extensive acute degenerative changes in the kidneys; the gastro-intestinal tract shows marked changes in the lining membrane which may be necrotic, sodden and in some places stripped away.

The cholera vibrios may sometimes be seen in enormous numbers in smears from typical stools. For a positive diagnosis, however, the organism must be cultivated. The usual method is to inoculate a 1 per cent peptone solution from the stool, incubating at 37° for from four to eight hours and sowing plates from the very surface of the liquid, either of gelatin or alkaline agar or both. The vibrios are 3μ to 5μ long by about 0.4μ wide, and are curved slightly like a comma or sometimes in a half circle. These comma forms are best seen in broth cultures. The ends are usually rounded. In young cultures the organisms are usually arranged singly, occasionally two may be found end to end in the form of an "S." There is no capsule, and no spore formation. There is a single terminal flagellum, and the organism is exceedingly motile. Does not stain as readily with the ordinary aniline dyes as many other bacteria. Fuchsin gives the best result. It is Gram-negative. The optimum temperature for growth is 37° with a minimum of 8° and a maximum of 42°. Plain agar—moist, shining, grayish yellow, and rather thin and transparent as compared with the colon type of colony. A rapid growth takes place in broth, causing a uniform clouding with a more or less well-developed pellicle. In gelatin plates colonies are visible in twenty-four hours and are round, even, and yellowish white, later they become irregular and their surface presents fine refractile granules; within forty-eight hours the colonies are found to be sinking into a small round pit due to liquefaction of the medium (Fig. 180). Concentric rings may appear as liquefaction progresses from day to day. In old cultures the liquefaction assumes a funnel or turnip shape with an air bubble at the surface due to evaporation. Growth in milk occurs without any visible change in the medium. At 37°, on potato, an abundant moist brownish growth. Blood serum is liquefied rapidly. The vibrios prefer the presence of oxygen, yet it is probable that organisms grow under practically anaerobic conditions in the intestine. The reaction of all media must be very distinctly alkaline and even very small amounts of acid are inhibitive. Neither gas nor acid is formed. The production of indol and the formation of nitrites from nitrates occurs regularly. The addition of sulphuric acid is sufficient to give the nitroso-indol reaction, which from its association with this bacterium has been called the cholera red reaction. No pigment is produced. Majority of freshly isolated cultures have hæmolytic powers. It is generally considered that there is only an endotoxin, but it is strongly asserted by some that a soluble toxin is formed. Thermal death-points are 60° for ten minutes, 95° to 100° for one minute. Vibrios are quite sensitive to low temperature and at most have been found viable in ice only after a few days. The vibrios are quite susceptible to the ordinary disinfectants.

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* Prepared by Edward Fidlar.

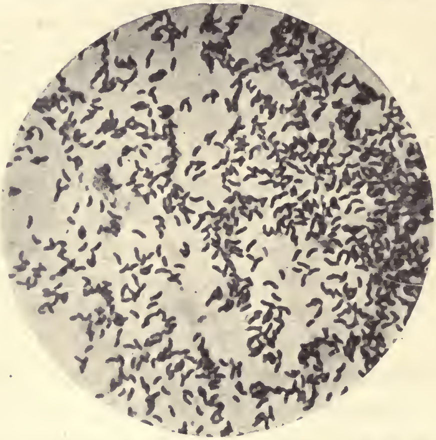

FIG. 179.—*Microspira comma.* × 1000. (*After Williams*)

The cholera organism gains entrance through the mouth.

Having succeeded in passing the acid secretions of the stomach the vibrios probably develop with great rapidity in the small intestine.

The peculiar conditions favorable to the development of the organism in the intestine are unknown. A previous gastro-intestinal disturbance is probably necessary even though slight.

The organisms have rarely been demonstrated in blood cultures. The gall-bladder gives the highest percentage of positive cultures.

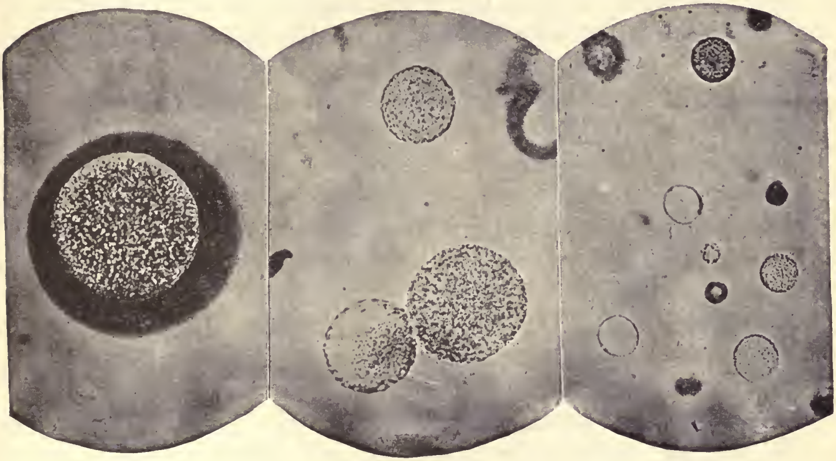

FIG. 180.—*Microspira comma.* Colonies on gelatin plates. *a,* Twenty-four hours old; *b,* thirty hours old; *c,* forty-eight hours old. (*After Fraenkel and Pfeiffer from Williams.*)

Highly lytic and agglutinating sera can be developed experimentally, but little or no antitoxic power can be demonstrated.

Protective inoculation has shown considerably more encouraging results than serum therapy.

The cholera vibrios are eliminated in the discharges. Water and uncooked food becoming contaminated with cholera excreta are the chief means by which the epidemic is spread, so that its epidemiology is similar to that of typhoid fever.

MICROBIAL DISEASES AS YET UNCLASSIFIED*

SCARLET FEVER, MEASLES, GERMAN MEASLES, DUKE'S DISEASE, SMALLPOX, CHICKENPOX, MUMPS†

These diseases constitute a group the actual biological causes of which are unknown, yet which show analogies to diseases the causes of which are known, so close as to make tenable the hypothesis that they are due to similar causes.

Mumps is in a class by itself, its characteristics, well known to the laity, marking it off from the others sharply. Like the others it is infectious; it is derived only from a preceding case; it has a more or less definite incubation period (*i.e.*, an interval between the date of infection and the first development of symptoms, during which ordinary health is enjoyed), and a prodromal stage (*i.e.*, a period in which fever, headache, and other more or less marked constitutional symptoms exist without any marked characteristic symptom). Then appears the swelling of the parotid salivary glands just in front of the ears with some pain. The symptoms usually amend after a few days and the patient goes on to full recovery. There is no rash nor any great disturbance of the intestinal tract or internal organs as a rule, although metastases, affecting the mammæ, ovaries or testicles develop at times; and secondary complications sometimes are found.

Smallpox and chickenpox together form a group quite often confused clinically, especially in the early stages and especially when smallpox is prevalent in mild form. They have incubation periods, approximating about twelve days, in smallpox varying little from this period, in chickenpox varying widely from it. Smallpox has rather severe prodromes, backache, headache, fever, and sore throat, the rash appearing on the third or fourth day. Chickenpox usually has light or no prodromes, the rash appearing on the same day or within twenty-four hours, as a rule. In both diseases the face, chest, back, arms, hands, legs, and feet are likely to show eruption, but chickenpox tends to show the greatest number of spots "under cover," *i.e.*, on the parts usually covered by clothing, while smallpox tends to show the majority upon the face, neck, arms, wrists, hands, legs and feet rather than on the body. The skin lesions themselves differ very markedly, the typical lesions of chickenpox being superficial, thin walled, high, rounded, and filled with clear liquid, those of smallpox being deep seated, tense, opaque, with a tough covering of epithelium. There are many other points of distinction, and any one familiar with the two diseases can hardly fall into error when dealing with typical cases at whatever stage they are encountered. To the layman's eye, however, the two are often indistinguishable.

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* Arranged alphabetically except group of diseases placed first

† Prepared by H. W. Hill.

Scarlet fever, measles, German measles, and Duke's disease are often likewise confused by the laity and even by physicians who have not had opportunities for extensive study.

German measles is clinically related to true measles somewhat as chickenpox is to smallpox, *i.e.*, they are wholly distinct diseases yet show characteristics easily confused on superficial consideration. Duke's disease is perhaps not a distinct entity; much has been said on this point and a satisfactory decision will probably never be reached until the causative agents have been found. It may be described briefly for clinical purposes as a variety of German measles having a scarlatiniform instead of a measly rash.

Scarlet fever has an average incubation period of about five days, or perhaps sometimes less. The prodromes are those usual to all these infections—headache, fever, and sore throat, but the latter is especially severe. Within twenty-four hours the rash appears usually on the chest first, a bright scarlet superficial punctate flush, extending rapidly over the body.

In measles the incubation period is longer, averaging nine or ten days, almost without any variation. The prodromes, headache, fever, and sore throat, are accompanied by very marked coryza and photophobia, catarrh and "cold on the chest."

The rash appears about the fourth day, appearing on the face and back but rapidly extending. It is darker, bluer, and deeper than the scarlet fever rash and unlike the latter is palpable. Koplik's spots appear on the buccal membrane early in the disease.

In German measles the prodromes are so indefinite that it is difficult to determine their length; very commonly the rash is the first thing noticed. It appears on the face, chest, back, and arms as a light subcuticular mottling (measles type) or a more uniform pink flush (scarlatiniform or Duke's type); with this rash the eyes show some injection and slight photophobia develops. The attack passes off quickly, without complications.

CANINE DISTEMPER*

This disease (Maladie des jeunes chiens; Fr.) is so widespread that the great majority of adult dogs may be regarded as having suffered from an attack and recovered. It is practically confined to very young animals and, so far as known, no species except dogs are susceptible. The disease is attended by more or less extensive coryza with a discharge from the eyes. There is an eruption on the skin and frequently nervous disorders of various kinds. The animal becomes emaciated and may die from bronchial pneumonia. No organism has been fully accepted as the cause of this disease. Carré has reported that he has succeeded in passing the infectious agent in nasal discharges through earthen filters, the filtrate reproducing distemper in characteristic form. Ferry has announced the discovery of an organism as the causal agent. Some attempts have been made to produce a protective serum.

CATTLE PLAGUE*

This disease (rinderpest), which is probably the severest and most contagious of all cattle diseases, is characterized by high fever and lesions of the intestinal tract. It does not exist in the United States but is found in Europe, S. Africa and Asia. Extensive outbreaks have occurred in the Philippine Islands. The cause of cattle plague has never been isolated and the indications are that it is caused by a filtrable microörganism. Cattle plague was the first disease in which the process of "hyperimmunization" was practiced. Immune cattle receive massive injections of blood from diseased cattle. After this treatment the blood serum of the immune is used to protect non-immunes. Enormous quantities of this serum are prepared and applied yearly by the British government in India.

CONTAGIOUS BOVINE PLEURO-PNEUMONIA*

This disease affects cattle only; it is highly infectious and produces an inflammation of the lungs and pleural membranes. Thirty years ago bovine pleuro-pneumonia was quite prevalent in the United States but has since been eradicated through the efforts of the Federal Bureau of Animal Industry in coöperation with State authorities. It still exists in European countries.

* Prepared by M. Dorset.

The microörganism of bovine pleuro-pneumonia is generally classed among the filtrable viruses, though unlike some organisms of that class it has been cultivated artificially and is just visible at a magnification of 2,000 diameters. The artificial cultivation of this virus was accomplished by Roux and Nocard through the use of the very ingenious "collodion sac method." A small amount of virus from a diseased cow was placed within a small thin-walled sac of collodion; after being hermetically sealed the sac was placed in the peritoneal cavity of a rabbit where it remained for several weeks. At the end of this time the unbroken sac was removed and the previously clear fluid within was found to be slightly opalescent. Microscopic examination revealed numberless minute motile bodies so small, however, that their exact form could not be determined. Later the organism was successfully cultivated outside of the animal body in a specially prepared bouillon. These cultures produced the disease when inoculated into susceptible cattle. When the virus is diluted it will pass through the Berkefeld and Chamberland F cylinders, but not through the Chamberland B cylinder.

Cowpox, Horsepox, and Sheeppox*

Variola refers to a condition or disease in man and animals, characterized by fever and the appearance of skin eruptions which successively assume the form of papules, vesicles and pustules. The disease is frequently found in the human species (smallpox), cattle (*variola vaccinia*, cowpox), horses (*variola equinæ*, horsepox) and sheep (*variola ovina*, sheeppox). It is possible that some other species may be susceptible.

On account of the fact that vaccination of man with virus from cases of cowpox affords remarkable protection against smallpox, it appears reasonable to believe that cowpox virus or smallpox vaccine is a modified form of smallpox virus. This fact, together with the occasional positive results of various experiments in which other species of animals have at times evidenced susceptibility to cowpox virus, strongly suggests the possible etiological relationship of the diseases in different species to each other and to smallpox in man. However, conclusive proof supporting this suggested relationship does not exist. The specific causative factor of smallpox or of cowpox is not known.

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* Prepared by W. E. King.

Cowpox is a very common disease, perhaps having been prevalent in England and Europe for centuries. Its presence has frequently been observed in various countries since 1796 when Jenner contributed to the world his important discovery relative to smallpox vaccination.

Many attempts have been made to isolate the causative factor of cowpox. Early investigators frequently secured mixed and pure cultures of various organisms, including different species of micrococci, streptococci and bacilli from vaccine lymph. None of these organisms were peculiar to the virus, and at present there exists no definite evidence that the infectious agent of vaccine lymph is of bacterial nature. Pfeiffer, Guanieri, Plimmer, Councilman, MacGrath, Brinckerhoff and others, after observing the presence of apparent cellular elements, or relatively large flattened bodies in vaccine lymph, have suggested the possible protozoan nature of the causative agent. Attempts have been made, with more or less success, to cultivate these bodies in collodion capsules in the peritoneal cavities of experimental animals. According to some investigators the virus has been passed through a Chamberland filter. The failure to discover the causative factor, according to the present methods, may be due to the inability of microbiologists to cultivate or stain the specific agent.

Cowpox is characterized by eruptions which usually occur on the skin of the teats and udder. The material contained in these pustules is transferred to other animals by the hands of the milker and through other possible means of dissemination. The chief channel of infection appears to be through an abrasion in the skin. The period of incubation of cowpox is about two days. The virus possesses relatively weak resistance to heat, light and chemicals. The control of the disease depends chiefly upon precautions relative to the transmission of the virus on the hands of the milker from infected to healthy cows.

Horsepox may be diagnosed by the appearance of the characteristic pustules usually upon the skin, nasal mucosa and buccal membrane.

Sheeppox is characterized by the presence of the typical skin eruptions, following a rise of temperature.

DENGUE*

This disease (break-bone fever) of man occurs in all parts of the world. It is characterized by a sudden attack, intense prostration and severe pains in the muscles and joints. The fever during the attack shows a characteristic curve. There is a sudden rise of and maintained temperature for several days. Then a remission and a second rise of temperature which is less than the first.

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* Prepared by M. Dorset.

Our knowledge of the cause of this disease rests chiefly upon researches of Ashburn and Craig.* These authors conclude that dengue is not contagious in the ordinary sense but that it is transmitted through the bite of the mosquito (*Culex fatigans*). No visible organism could be demonstrated in either fresh or stained specimens of blood from patients affected with dengue although such blood was capable of producing a typical attack of dengue when inoculated intravenously into healthy men. The authors likewise show that blood from a case of dengue retained its infectiveness after passage through a filter made of diatomaceous earth.

FOOT-AND-MOUTH DISEASE†

Foot-and-mouth disease is primarily a disease of cattle, though the other domestic animals and man may be attacked. The disease is very contagious and is characterized by the eruption of vesicles in the mouths, on the udders and on the skin surrounding the hoofs of cattle. It is very prevalent in European countries. There have been three outbreaks in the United States all of which were promptly eradicated by vigorous repressive measures instituted by the Federal authorities.

The cause of this disease is an invisible microörganism which exists in the lymph from the vesicles which form in the mouths and on the feet of cattle. This virus has never been cultivated artificially. It passes through the Berkefeld cylinder but not through the finer-pored Kitasato filters; it is quickly destroyed by formaldehyde, carbolic acid and similar disinfectants.

The disease is readily transmitted from one animal to another by contact and the contagion may persist for some time in the manure, or straw from infected stables. The milk of infected cows has been said to produce the disease in children.

Animals which recover from an attack remain immune for a short time only; it is therefore not surprising that no satisfactory means of artificial immunization has been devised.

* Ashburn, P. M. and Craig, C. F.: Jour. Inf. Dis., Vol, IV, p. 440, 1907.

† Prepared by M. Dorset.

FOWL PLAGUE*

This disease (Hühner Pest; Ger.: Peste aviaire; Fr.) of fowls, which is to be distinguished from chicken cholera, is not known in the United States, but has caused extensive losses of fowls in Europe, particularly in Italy. Affected chickens cease eating, the feathers become ruffled and the comb darker in color. The lesions found at autopsy are not constant, but a pericarditis is usually seen. There may be, also, congestion of the lungs, liver, and kidneys. The intestinal lesions are not as marked as is the case in chicken cholera.

Fowl plague has been shown to be due to an invisible microörganism which is present in the heart blood and in practically all of the organs of the body. Most fowls are susceptible; guinea-pigs and mice are refractory to the disease. The virus passes through Berkefeld and Chamberland F cylinders; it is quite resistant to drying but is destroyed by an exposure of half an hour to a temperature of 60°. Several authorities have passed the filtered virus through four or more hens successively, thus demonstrating positively that the filtered virus is capable of multiplication.

HOG CHOLERA*

The first recorded outbreak of hog cholera in the United States occurred in Ohio in the year 1833 and it now exists in practically all sections of this country. Hog cholera is most prevalent in the late summer and fall, although outbreaks are reported at all seasons of the year. All races of hogs are susceptible and the average mortality is about 80 per cent. In the United States alone the losses from hog cholera are estimated to average at least $15,000,000 annually. Hogs only are attacked. This disease is supposed to have been introduced into the United States through the importation of hogs from Europe, where it is known under the names "swine fever" (Br.), "schweinepest" (Ger.), and "peste du porc" (Fr.).

The essential features of hog cholera may be briefly summarized as follows: Extreme contagiousness. Symptoms of severe illness accompanied by fever, loss of appetite, weakness and diarrhœa. Hæmorrhagic lesions in the various organs and lymphatic glands and round button-like ulcers in the large intestine. Immunity in hogs which recover.

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* Prepared by M. Dorset.

The etiology of hog cholera has long been the subject of scientific controversy, but it is now generally acknowledged that the cause of this disease is a filtrable microörganism which exists in the blood, the internal organs, and the urine of infected hogs. The fact that this disease is caused by a filtrable microörganism was demonstrated as follows:*

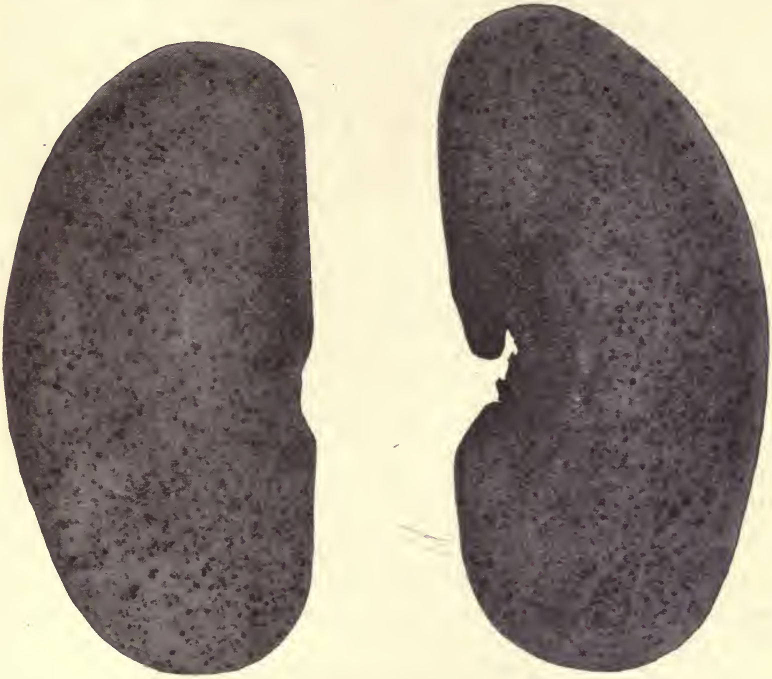

FIG. 181.—Hæmorrhagic points on kidneys of hog-cholera hog. (Original.)

The blood serum of hogs infected with hog cholera acquired in the natural way is very infectious for non-immune hogs, the disease being readily transmitted by the subcutaneous injection of small amounts. The disease produced by this subcutaneous injection is identical in all respects with the disease as it occurs in nature. If, now, this infectious serum is diluted with normal salt solution or with ordinary bouillon (1 to 10) and passed through either a Berkefeld or Chamberland filter, the filtrate, though free from all visible microörganisms, still retains the power to produce hog cholera by subcutaneous injection. The disease which is produced in this manner by the filtered hog cholera serum is identical in all respects with the disease produced by the unfiltered serum and also with the disease as it occurs in nature. The hogs which receive the filtered serum present the symptoms and lesions of hog cholera. The disease set up in this manner is very contagious and hogs which recover from the inoculation of filtered serum are thereafter immune against hog cholera. By repeated inoculation and filtration this virus may serve to infect successively a large number of hogs.

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* Bulletin 72, Bureau of Animal Industry, U. S. Dept. Agriculture, 1905.

The invisible virus of hog cholera, in view of its ability to pass through the Chamberland B filter, must be regarded as one of the smallest of the filtrable microörganisms. It has never been cultivated artificially, hence, aside from its disease-producing qualities, we have little knowledge concerning it. We do know, however, that the virus is quite resistant to such common disinfectants as carbolic acid and bichloride of mercury and that it is quickly destroyed by a 3 per cent solution of *liquor cresolis compositus* (U. S. P.) as well as by a 5 per cent solution of antiformin. When preserved in sealed glass bulbs in a cool dark place, the virus retains its activity for six months or longer. Rabbits, guinea-pigs, and other small animals are entirely insusceptible to inoculations of the filtered virus in amounts which would prove fatal to hogs.

The virus of hog cholera is known to be thrown off from the body through the urine, the fæces and the eye and nose secretions. Therefore any agency which would serve to carry a particle of dirt from infected hog yards might be the means of disseminating the virus. As many sick hogs find their way to the public stock yards through shipment by rail, all stock cars and stock yards are to be regarded as permanently infected. It appears to be impracticable to prevent the spread of the disease by methods of quarantine and disinfection alone, owing to the impossibility of enforcing such measures thoroughly. It has recently been found that a protective serum against hog cholera may be produced by "hyperimmunization." The process consists in giving immune hogs large doses of blood taken from hogs sick of hog cholera. As a result of this blood treatment their serum acquires the power to protect non-immunes. Injections of serum from hyperimmunized animals confers a passive immunity, while the simultaneous injection of serum with a small amount of virus produces an active immunity.

Bacillus Choleræ Suis (*B. suipestifer*).—No description of the etiology of hog cholera would be complete without a reference to this bacterium which was long regarded as the cause of hog cholera. It is found after death in the blood and organs of the majority of hogs affected with hog cholera and in this rôle of secondary invader it no doubt tends to increase the mortality from the disease. *B. choleræ suis* is a small, very actively motile, non-spore-bearing bacillus with rounded ends, and stains readily with the ordinary aniline dyes. It does not stain by Gram's method. This organism is easily cultivated on the ordinary media; gelatin is not liquefied; milk is not coagulated but acquires an acid reaction at first; this changes after a week or more to an alkaline reaction. Gas is produced in bouillon containing dextrose, but lactose and saccharose are not affected. Rabbits and guinea pigs succumb within four to ten days to small doses of this organism. Hogs are much more refractory. It is only after the administration of large doses that they show any symptoms of illness following subcutaneous injections. By feeding pure cultures of *B. choleræ suis* or by injecting these intravenously a considerable number of hogs will succumb and at autopsy present lesions which correspond quite closely to those seen in naturally acquired cases of hog cholera. There are, however, certain important differences between the disease produced by *B. choleræ suis* and the natural disease hog cholera. For example, hogs infected with *B. choleræ suis* do not transmit the disease to other hogs by contact. The blood of hogs infected with *B. choleræ suis* does not produce disease when injected subcutaneously into other hogs, and, in addition, hogs which recover from illness produced by injections or feedings of pure cultures of *B. choleræ suis* have no immunity against the natural disease hog cholera.

Horse Sickness*

This disease affects the equine species only and appears to be confined to South Africa. It is most prevalent in summer and appears to be transmitted by the bite of an insect, as it is not contagious but may be communicated to susceptible horses through blood inoculations. This disease manifests itself by producing severe inflammatory changes in the lungs and in the tissues of the head and neck and is attended by a high mortality. No visible organism has been found which will produce horse sickness and as McFadyean and Nocard have shown that the virus is capable of passing through the finest bacteria-proof filters, this disease is probably caused by an invisible microörganism. Blood containing the microörganisms of horse sickness may be kept in sealed bulbs in the dark at room temperature for more than two years without losing its infectiveness. The virus is quite resistant to drying and may survive heating for ten minutes at a temperature of 75°.

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* Prepared by M. Dorset.

Infantile Paralysis*

As indicated by its name, this disease (epidemic poliomyelitis) is usually seen in children. It has long been known to exist in both Europe and America, occurring generally in sporadic form. During the last decade, however, its prevalence has greatly increased and a number of well-defined epidemics have been reported. Though the character of this malady long ago led to the belief that it was caused by a microörganism, this fact was not definitely proven until the year 1909 when Landsteiner and Popper in Germany, and Straus and Huntoon and Flexner and Lewis in the United States, succeeded in transmitting the infection to monkeys. So far as is now known, none of the lower animals except monkeys are susceptible.

The symptoms and effects of infantile paralysis are extremely variable. Paralysis is by no means constant, many cases being very mild and thus possibly escaping detection. In the severer forms of the disease paralyses of various types and degrees are seen. When recovery takes place the paralysis may appear to improve only to be followed by atrophy of certain groups of muscles, resulting in deformity and permanent lameness. These effects are caused by the destruction of certain nerve centers in the spinal cord.

As stated above, the microbial origin of infantile paralysis was first demonstrated by the inoculation of monkeys, Flexner and Lewis having successfully carried the infection through a long series of monkeys by successive intracranial injections of an emulsion of the spinal cord taken from infected animals. The microörganism passes through the Chamberland and Berkefeld filters with little or no loss in disease-producing power. "Flexner and Noguchi, employing the technic previously used for cultivating pathogenic spirochætes, have succeeded in obtaining from infected tissues cultures of a minute round organism which they believe to be the cause of infantile paralysis." The virus withstands freezing or drying for long periods of time but is quickly destroyed by heating at a temperature of 50°. It is likewise quickly killed by the ordinary disinfectants. Monkeys may be infected by the subcutaneous, intraperitoneal, intravenous, or intracranial injection of material from an infected spinal cord, but attempts at infection through feeding have been unsuccessful. The virus appears to be eliminated from the body through the nasal mucous membranes.

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* Prepared by M. Dorset.

It appears probable that one attack of the disease protects from a second attack. No cases of a second attack have been reported. Furthermore, monkeys which have recovered from the infection appear to be entirely immune as shown by Flexner. Active immunity in monkeys has been established by repeated infections of gradually increased amounts of the virus. The blood of human beings and of monkeys that have recovered from an attack of the disease is capable of neutralizing a certain amount of the virus. This protective quality of the blood serum may be increased by repeated inoculations of virus, and infection in monkeys can be prevented by injecting simultaneously the virus into the brain and the serum into the sub-arachnoid space. The serum treatment of this disease is, however, not developed to such a state that it can be regarded as of practical use.

PELLAGRA*

Pellagra is a disease of man characterized by the annually recurring manifestation, each spring or summer, of erythema on the backs of the hands and forearms and sometimes on the face and neck, feet and ankles, coupled with digestive disorder and more or less well-marked mental disturbances. During the winter the signs of the disease usually disappear.

At present there are two main groups of theories concerning the causation of pellagra, each of which includes a multitude of hypotheses. According to one group of theories, pellagra is a food poisoning due to eating maize (Indian corn); according to the other, pellagra is a specific infectious disease not necessarily associated with the ingestion of corn. None of the theories concerning causation is supported by conclusive evidence. The evidence against the corn theory marshalled by Sambon and others has greatly weakened the almost general belief in this theory which formerly obtained. Some prominent zeists have recently shown a tendency to ascribe pellagra not essentially to the use of maize but to a supposed deficiency or lack of a necessary something in the diet. This change of opinion has been caused in part by the failure of the maize theory when put to the test of actual observation and in part by an eager application to pellagra of the facts learned in the study of another disease, namely, beriberi. To the writer it seems very improbable that this new phase of the dietary theory will survive as long as has the maize theory proper, although it has received enthusiastic support from the U. S. Public Health Service.

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* Prepared by W. J. MacNeal.

According to the second theory, pellagra is a specific infectious disease, in which poor nutrition is one of the important predisposing factors. The epidemiological study of pellagra, as it has developed and spread in certain parts of the southern United States, has brought to light evidence of its infectious nature which, to the writer, seems very convincing. The same investigations have also strongly suggested that the infection is intestinal and transmitted in much the same way as is typhoid fever. A specific microbic cause of pellagra has not been identified.

The final decision in regard to the essential nature of pellagra must therefore await further research. Certain facts in regard to the disease are, however, well established. In the first place modern students agree that the ingestion of maize is not essential for the production of pellagra. It occurs in persons who have not eaten this food. The prevalence of pellagra, especially in institutions, bears a very definite relation to the deficiency of animal protein in the diet, as was first pointed out in this country by the Illinois State Pellagra Commission. This commission observed that pellagra diminished in the Peoria and Dunning institutions coincidently with an increase in meat supply, while at the Elgin Hospital the number of pellagrins increased with a decrease in the amount of meat provided per capita. This commission made a specific recommendation to the Governor of Illinois that "as a prophylactic measure the animal protein content of the State Hospital dietaries be increased." This finding of the Illinois Commission has been confirmed by subsequent investigators. Thus the Thompson-McFadden Pellagra Commission found, in 1913, that the individuals in Southern cotton-mill villages, in whose families milk was an article of daily use, were distinctly less subject to pellagra than their neighbors who did not use milk. More recently the dietary studies of the U. S. Public Health Service have confirmed these findings, especially in respect to the value of milk. However, the study of actual dietaries of pellagrins and their comparison with dietaries of other people in the same district has pointed clearly to the conclusion that there is no single element in these diets nor any group of elements, the inclusion or exclusion of which can be regarded either as the cause or as a certain preventive of pellagra.

The Illinois Commission, in 1911, placed as its first conclusion "According to the weight of evidence pellagra is a disease due to infection with a living microörganism of unknown nature." The Thompson-McFadden Commission in 1913 found that "new cases of pellagra originated almost exclusively in a house in which a preëxisting pellagrin was living, or next door to such a house, suggesting that the disease has spread from old cases as centers." Such spread was most rapid where insanitary methods of sewage disposal were in use. In a later report, 1917, this commission confirmed these conclusions and presented the details of an extensive experiment conducted in the community of Spartan Mills, Spartanburg, S. C., where, by replacing the insanitary surface privies with an efficient water carrier sewer system, one of the worst pellagra foci was transformed into a community in which the disease no longer spread. These observations have, in all essentials, been confirmed by Jobling, Petersen and their co-workers, (1916, 1917) at Nashville, Tenn., who found pellagra to be "practically a disease of the unsewered city areas, a family disease or almost as frequently a disease of the house next door."

These investigations have, therefore, not only shown that endemic pellagra may be alleviated by improvement in dietary and that its further spread can be effectively checked by sanitary measures directed to the proper disposal of sewage, but also have pointed to the intestinal tract as the location in which the parasitic cause of pellagra is to be sought.

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Rabies*

Lyssa or Rabies, the madness of dogs, was recognized as a definite disease of animals and man by the peoples of ancient times. The disease is generally distributed throughout the civilized world except in those places where special measures to stamp it out have been enforced. It does not arise spontaneously but is an infectious disease transmitted from animal to animal. Rabies is primarily a disease of wolves and dogs, and the bite of a mad dog is the most frequent cause of the disease in other animals and in man. It is not uncommon in horses and cattle, and all mammals appear to be susceptible to it.

In animals inoculated by injection of the most virulent virus (fixed virus) directly into the brain, the symptoms of rabies appear in four to six days and death usually occurs on the seventh day. Accidental inoculation by the bite of a rabid animal (street virus) rarely causes the symptoms to appear before three weeks, and the onset may be delayed for six months or a year. Not all persons or animals bitten by rabid animals take the disease; probably not more than one in four or five. This variability depends upon several factors, the most important ones being the virulence and the amount of disease virus, and the part of the body into which it is introduecd. Bites upon the face or hands, because of the rich *nerve supply* of these regions and the lack of protection by clothing, are likely to result in rabies sooner than bites elsewhere.

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* **Prepared by W. J. MacNeal.**

After the disease has developed, death is inevitable. In all animals the symptoms are those of a nervous disorder. At first there is excitation, and this is followed by paralysis and death, the relative length of the two stages varying in different animals. In the dog the disease runs its course in six to eight days. It begins with altered behavior of the animal, itching of the infecting scar, changed appetite, and slight fever. The dog swallows grass, stones, and pieces of wood. As the stage of excitement becomes more fully developed, the animal may run away and may travel fifty miles or more, snapping and biting from time to time, as the fits seize him, everything in his path. Finally the excitement is succeeded by paralysis, beginning in the lower jaw, which hangs down. Then the hind legs fail, and soon the dog, no longer able to drag himself along, lies completely paralyzed, greatly emaciated, and soon dies. In the rabbit the stage of excitement is hardly noticeable, but the animal passes quickly into the paralytic stage, dying after two or three days. This type of paralytic rabies sometimes occurs in dogs, but is more commonly observed in herbivorous animals.

In man there is a first psychical change, irritation in the scar of the infecting wound and rise of temperature. The first diagnostic symptom is usually a sudden spasm of the pharynx upon an attempt to swallow water. This convulsive seizure is repeated upon every attempt to drink, and soon even the sight of water or the thought of it brings on the attack. The cramps extend to other muscles of the body, and the patient may die in a convulsive seizure, or may pass into the succeeding paralytic stage and die peacefully. The dread of water which is often so prominent a symptom in man has given the name of hydrophobia to the disease. Consciousness and general intelligence are not particularly affected. The duration of active symptoms of the disease is from three to six days.

Rabies can be transmitted with certainty by injecting a small amount of emulsified spinal cord of the rabid animal into the brain of a rabbit or guinea-pig. Inoculation under the skin is not quite so certain, and inoculation into the blood stream, or by feeding, generally fails to transmit the disease. When first removed from a rabid dog, the virus (street virus) kills rabbits in from two to four weeks, but after repeated transfer from rabbit to rabbit in series, the period of incubation is shortened until death occurs quite regularly in six or seven days after inoculation. Beyond this there is no further increase in virulence for rabbits, and this six- or seven-day virus is called the "fixed virus."

The localization of virus in the body of the rabid animal has been worked out by experimental inoculations. The central nervous system is always virulent, as are also the salivary glands and the saliva. The peripheral nerves frequently contain the virus, less commonly other glands and secretions such as the tears, urine and milk. The virus has never been found in the liver or spleen, or in the blood. Under ordinary conditions, the chief source of danger is the saliva of the rabid animal, especially when this is introduced into a wound.

Rabies may be recognized in a dog in one of the three ways: observation of the course of the disease; autopsy; inoculation of test-animals and observation of the course of the disease in them. If the suspected dog is chained or caged, the question of rabies may be settled in a few days, for, if mad, the raging stage will be succeeded by the characteristic paralysis and death. If the dog has already been killed, a careful autopsy may show the absence of normal food from the digestive tract and the presence there of abnormal ingested material, highly suggestive of rabies. Microscopic examination of the central nervous system is, in the hands of an expert, a reliable method of diagnosis, which in this case depends upon the finding of the characteristic Negri bodies in the specimen. For confirmation of the diagnosis, a portion of the brain or spinal cord, removed without contamination, should be injected into the brain of test animals, and the effects observed. This last test carried out by experienced observers is justly regarded as the most trustworthy of all.

THE NEGRI BODIES.—The peculiar bodies found by Negri in the central nervous system of rabid animals seem to occur invariably and exclusively in this disease, and it is probable that they represent stages in the development of the infectious agent. These bodies are especially numerous and most easily demonstrated in the Ammon's horn of the brain in cases of the natural disease in dogs (street rabies). Excellent results may be obtained by the method of Lentz.*

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* Lentz, Otto, Ein Beitrag zur Faerbung der Negrischen Koerperchen. Centralbl. f. Bakt. etc., I Abt., Bd. XLIV, pp. 374–378.

Transverse sections, 2 to 3 mm. in thickness, of the Ammon's horn of the suspected brain are hardened in acetone at 37° for one hour, then transferred to melted paraffin (melting point 55°) in the paraffin oven at 58° for one and one-half hours and embedded. Sections, 2 to 3μ in thickness, are then cut with the microtome, floated upon lukewarm water and mounted upon perfectly clean flamed glass slides. The excess of water is carefully removed with filter paper and the slides are then completely dried on a warm plate at 45° or in the incubator at 37°. The sections adhere perfectly as a rule and are dry enough to proceed with after ten to fifteen minutes. The slides are next transferred to xylol to remove the paraffin and thence to absolute alcohol. The staining procedure is as follows:

1. One minute in alcoholic eosin.
   Eosin extra B-Hoechst, 0.5.
   Alcohol, 60 per cent, 100.0.
2. Wash in water.
3. One minute in Loeffler's methylene blue.
   Saturated alcoholic solution of methylene blue, B-Patent Hoechst, 30.0.
   Potassium hydroxide solution, 0.01 per cent 100.0.
4. Wash in water.
5. One minute in Gram's solution.
   Iodine, 1.0.
   Potassium iodide, 2.0.
   Distilled water, 300.0.
6. Wash in water.
7. Methylic alcohol until the preparation becomes entirely red.
8. Wash in water.
9. Loeffler's methylene blue again for thirty seconds.
10. Wash in water.
11. Dry carefully by pressing with filter paper upon the preparation.
12. Differentiate in alkaline alcohol until only a weak eosin color remains in the preparation.
    Absolute alcohol, 30.0 c.c.
    Sodium hydroxide, 1 per cent solution in absolute alcohol, 5 drops.
13. Differentiate in acid alcohol until the collections of ganglion cells in the gray matter are still faintly blue while the rest of the section is free from blue (macroscopic).
    Absolute alcohol, 30.0 c.c.
    Acetic acid, 50 per cent, 1 drop.
14. Wash quickly in absolute alcohol.
15. Xylol.
16. Balsam and cover-glass.

Steps 5 to 9, inclusive, may be omitted to save time at some sacrifice in the final result. The Negri body is stained pink with blue granules in its interior. The nerve cells are stained pale blue.

Although sections are most satisfactory for diagnostic purposes and especially to show the relation of the Negri bodies to the ganglion cells, it is usually possible to recognize the Negri bodies in smears, after a little experience. For this purpose a portion of the gray matter of the Ammon's horn is crushed by gentle pressure between two perfectly clean flamed slides and spread upon them by carefully slipping the slides apart. The moist smears are at once fixed in methyl alcohol for one minute, then washed in absolute ethyl alcohol, whereupon they are ready to be stained by the procedure outlined above.

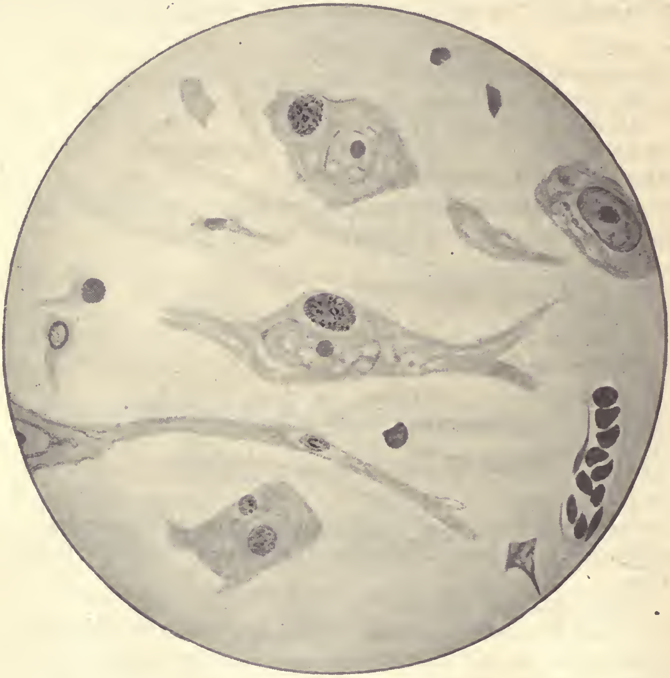

FIG. 182.—Section through the *cornu ammonis* of brain of a rabid dog; stained by the method of Lentz. Five Negri bodies of different sizes are shown, enclosed within the ganglion cells. The smallest contains only three minute granules. (*After Lentz, Centralbl. f. Bakt.* 1907, Abt. I, Vol. XLIV, p. 378.)

The Negri bodies (Fig. 182) appear as round or somewhat triangular structures, for the most part inside the ganglion cells. Their size varies considerably, from 1μ to 27μ in diameter, the majority measuring about 5μ. In the interior of the Negri body, smaller structures of variable size and number can be seen. These granules may be differentially stained as in the Lentz method. Some careful students of rabies regard the Negri bodies as protozoa and consider them to be the infectious agent. Proof of this belief is still lacking inasmuch as it has not yet been conclusively shown that they are actually living organisms.

A wound infected by a rabid animal should be thoroughly cauterized, under anæsthesia if desired, at the earliest possible moment, and this cauterization should not be omitted even if twenty-four hours have elapsed. Cauterization cannot be relied upon to prevent the development of rabies, but it does serve to prolong the incubation period. The Pasteur treatment should then be instituted as soon as possible, and it has proved to be practically an absolute preventive, provided the incubation period of the disease is sufficiently prolonged for the treatment to become effective, and this is usually the case. The treatment consists in the daily subcutaneous injections of altered fixed virus for a period of about three weeks, and is most effectively given at Pasteur Institutes devoted especially to this work. Valuable animals as well as man may be successfully treated in this way.

The general prevention of rabies depends almost solely upon the efficient control of all dogs in a community. General muzzling, strictly enforced, is a certain preventive of rabies, and in countries where this is done rabies is practically unknown.

SWAMP FEVER*

This is a comparatively new disease of horses so far as definite information is concerned, but is in reality an old disease that has been described under a variety of names for many years. It is known by various names as infectious anæmia, malarial fever, horse typhoid, "plains" paralysis, and pernicious anæmia, and has been recognized in many portions of the United States and Canada.

This disease is usually of chronic type, but acute cases have been reported. There is usually a long illness extending from a month to a year or more, and marked by periods of fever and debility, alternating with periods of apparent recovery. The phase of apparent illness is characterized by fever, general weakness, and staggering gait, and the disease terminates fatally, as a rule. Some cases undoubtedly terminate as "carriers." The peculiar features of the disease are the alternating periods of illness and recovery, unthriftiness in spite of unusually good appetite, pallor of mucous membranes, dropsical swellings of the belly and limbs.

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* Prepared by M. H. Reynolds.

It has been satisfactorily proved that swamp fever is caused by filtrable virus present in blood and urine and which is quite resistant to drying, putrefaction and low temperatures.

Under artificial inoculation with blood, the period of incubation varies from ten to forty days. The natural method of infection is unknown, but there are reasons for believing that infection does not easily occur by way of the respiratory or the digestive organs. The disease is apparently not communicated by simply stabling diseased animals with healthy animals. A Japanese commission has incriminated certain biting flies.

Distribution in the body is very general, as shown by the wide distribution of characteristic lesions, and as shown by the fact that the blood is infectious.

The virus which causes swamp fever reduces greatly the number of red blood corpuscles and also produces local hæmorrhages which are most frequently small and sharply defined. The reduction of red blood cells produces marked pallor, and there gradually develops noticeable emaciation.

Post-mortem lesions in many cases are slight. The hæmorrhages may involve subcutaneous and intermuscular tissues, liver, spleen, kidneys and lymph glands and are rather common on the lungs and heart. Any of the abdominal organs may show the characteristic hæmorrhages. The bone marrow has been reported in some cases as distinctly changed in color, the yellow marrow of long bones becoming dark red. In some cases the liver shows enlargement, degeneration and necrosis.

TYPHUS FEVER*

Typhus fever (ship fever, jail fever) has been known to exist for centuries but until very recently we have been without precise knowledge concerning its cause. Typhus is found in all parts of the world; it affects man only and is characterized by a high fever and an eruption on the skin. The course of the disease is limited and lasts for only about twelve days. In the years 1909 and 1910 Nicolle, working

* Prepared by M. Dorset.

in Tunis, and Anderson and Goldberger, and Ricketts and Wilder working in Mexico, showed that typhus is communicated from man to man by means of the body louse (*Pediculus vestimenti*), and that the disease is not contagious in the ordinary sense of the word. Nicolle states that after biting a typhus fever patient the louse cannot convey the infection until the fourth day thereafter and that it loses this power after the seventh day. This indicates a similarity between the micro-organisms causing yellow fever, malaria and typhus. The disease may be communicated to monkeys by subcutaneous inoculations of blood from a typhus fever patient. The virus may be transferred from one monkey to another indefinitely. In monkeys recovery from severe attack produces a firm immunity. Plotz* has isolated a small Gram-positive bacillus which he believes to be the cause of the disease. Attempts to pass the virus through filters have been unsuccessful with the possible exception of certain experiments by Nicolle. The virus is destroyed by heating from 50 to 55°.

YELLOW FEVER†

Yellow fever is an acute infectious, non-contagious disease of man which is seen in tropical and sub-tropical countries, particularly the West Indies, South America, and the west coast of Africa. The most notable symptoms of the disease are fever, jaundice, and hæmorrhages from the mucous membranes, this latter resulting in severe cases in what is known as "Black Vomit," which consists chiefly of extravasated blood which has been changed to a brown or black color by the action of the gastric juice.

Prior to the brilliant researches of Walter Reed and his associates on the United States Army Commission in the year 1900, it was generally believed that yellow fever was contagious, and that the disease was transmitted directly from infected to non-infected individuals, and furthermore that the clothing, bedding, and all materials which came into contact with the infected subject were capable of transmitting the disease. Reed and his associates, during the American occupation of Cuba, secured a number of volunteer subjects to serve the Commission in its studies. This Commission demonstrated positively that yellow fever was not transmitted to man in any other way than by the bite of a particular mosquito, *Aëdes* (*Stegomyia*) *calopus* (Meigen). These mosquitoes were allowed to bite patients suffering from yellow fever at different stages of the disease. Subsequently these same mosquitoes were allowed to bite healthy men at different periods of time following their application to the infected individual. It was proved that the mosquito, in order to be capable of conveying the disease, must bite an infected individual during the first three days of the fever and at least twelve days must elapse thereafter before the mosquito is capable of transmitting the disease to a susceptible individual.

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* Plotz, Jour. Inf. Dis., July, 1915.

† Prepared by M. Dorset.

Quite recently Noguchi* has isolated from cases of yellow fever a spiral microörganism which he calls *Leptospira icteroides*. He has grown this microörganism in pure cultures which proved pathogenic for guinea-pigs. Infected guinea-pigs develop symptoms and lesions resembling those of yellow fever in man. *Leptospira icteroides* is capable of passing through Berkefeld filters. The present indications are that it is the cause of yellow fever.

DISEASES CAUSED BY PROTOZOA†‡

RHIZOPODA (von Seibold)

The amœbæ are the most important of the parasites belonging to the rhizopods. Various species of amœbæ are parasitic in the intestines of cattle, horses, mice, frogs and fish as well as human beings and most of them, like *Entamœba coli* of man, are harmless. One species, *Entamœba histolytica*, produces a very severe disease of man. *Entamœba meleagridis* is the cause of a fatal disease of turkeys (page 879). *Entamœba gingivalis* (*buccalis*) is a parasite which is frequently found in a diseased condition of the gums characterized by peridental abscesses but is also frequent in apparently healthy mouths. Amœbæ have also been found in purulent and serous fluids from the chest and abdomen as well as in urine. The parasitic species lack the contractile vacuole which is a feature of the free living species that are commonly encountered so that it is not difficult to distinguish the two types.

AMŒBIC DYSENTERY

Entamœba histolytica—Schaudinn, 1903

Syn.: *Entamœba tetragena*—Viereck, 1907

Distribution.—Amœbic dysentery occurs most frequently in tropical, or sub-tropical, countries, but cases of it occasionally occur in Great Britain, in Central Europe, and in the United States.

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* Noguchi, H., Etiology of Yellow Fever, Jour. Exp. Med., Vol. XXIX, No. 6, *et seq.*, 1919.

† Diseases arranged generically.

‡ Prepared by J. L. Todd.

Intestinal amœbæ; Entamœba coli Lösch (Fig. 183) and *Entamœba histolytica* are both parasites in the human intestine. They measure from 15μ to 30μ in diameter and, when examined in freshly passed fæces, may be seen in active motion. Their cytoplasm contains a nucleus, vacuoles, and food particles. Both may multiply by binary and by multiple division; the appearance of certain of the encysted forms in both species indicates a process of autogamy. Both of the parasitic amœbæ of the human intestine produce a characteristic number of daughter amœbæ in the course of their multiplication. *E. coli* commonly divides into eight small amœbæ so that these organisms may present any number of nuclei below this number and occasionally they contain several more. The encysted forms of this species also divide into approximately eight small amœbæ. In *E. histolytica* the number produced as the result of division is more regular; it is almost invariably four in the division both of the motile trophozoites and of the encysted forms. The character of the division thus furnishes the most certain criterion in differentiating the two species. Multiplying forms are not always readily found, however, and it is necessary to take other characteristics into consideration. The non-pathogenic species (*E. coli*) is more sluggish in its movements, is generally larger, dull grayish in appearance, and has no sharp differentiation into ectoplasm and endoplasm. *E. histolytica* is active, of a greenish hue and the ectoplasm is well defined and very clear in portions extruded as pseudopods. The nucleus in the harmless species, commonly centrally situated, is larger and shows a larger amount of chromatin. The nucleus of the dysentery amœbæ is smaller,

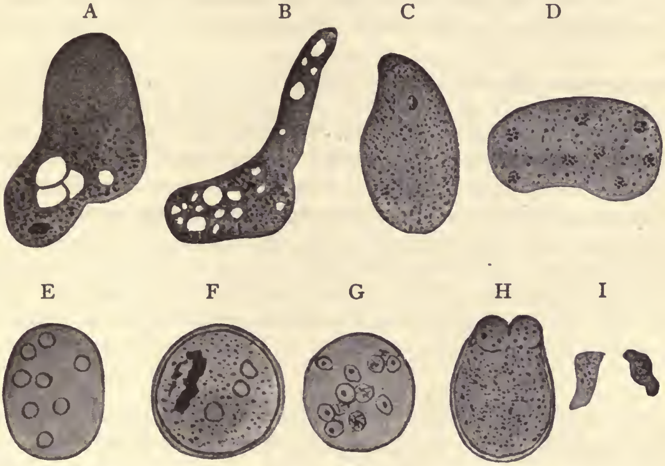


FIG. 183.—*Entamœba coli* Lösch 1875. *A-C*, various forms of motile amœbæ *D*, the 8-nuclear stage; *E-G*, cysts with nuclear fragments; *H*, bursting cyst; *I*; young motile amœbæ. (*After Casagrandi and Barbagallo, from Doflein.*)

poorer in chromatin and commonly peripherally situated in the cytoplasm. This species also devours red corpuscles in large numbers but the absence of these in intestinal amœbæ is not sufficient basis for considering them to belong to the harmless species. The cysts are passed in the fæces; and it is through the ingestion of food or drink, contaminated by encysted amœbæ, that infection is accomplished. If unencysted amœbæ are swallowed, they are digested by the acid juices of the stomach, whereas encysted amœbæ pass through the stomach unaltered and become active in the alkaline contents of the intestine. The dysentery amœba is pathogenic to certain lower animals, and kittens have been found to be most favorable for the experimental production of the disease. Monkeys are also susceptible to a certain extent.

Entamœba histolytica may be present in an intestine for months without marked symptoms resulting. It may, however, enter one of the glands of Lieberkühn and pass through it into the submucosal layer of the intestine. Bacteria accompany the amœbæ and they, with the bacteria, cause an ulcer which spreads through a local destruction of the submucosa, and undermines the mucosal layer of the intestine. In severe cases, when the ulcers have spread widely, large areas of the mucosa may be sloughed off. The amœbæ lie at the edge of the ulcer and cause it to enlarge by working their way into sound tissue; once an ulcer is started, it is not impossible that *Entamœba coli* as well as the dysentery amœbæ may be found in it. The latter live upon the red cells or fragments of intestinal cells. In chronic cases, the wall of the intestine becomes greatly thickened.

Ulcers caused by amœbæ are almost always situated in the large intestine; consequently, the symptoms of amœbic dysentery are those of inflammation of that part of the body. There is usually abdominal pain, accompanied by the passage of frequent, blood-stained stools with mucus. The infection may, however, be accompanied by no marked symptoms and there may be no diarrhœa. There are usually developed more general symptoms, such as fever and loss of flesh. The onset is frequently very gradual and insidious and the disease runs a chronic course. If amœbic dysentery causes death, it usually does so by perforation of the bowel with resulting peritonitis, by hæmorrhage from the erosion of a blood vessel, or by producing an abscess of the liver. Liver abscesses occur not infrequently in amœbic dysentery.

Amœbic dysentery is cured with difficulty although *emetine*, a product isolated from ipecac, has recently been found of great value. Since the encysted amœbæ are killed by heat, dysentery can be avoided by eating and drinking only foods and liquids that have been cooked.

ENTERO-HEPATITIS OF TURKEYS

Entamœba meleagridis—Smith, 1895

Entero-hepatitis, or black-head, of turkeys is caused by *Entamœba meleagridis*. The disease is widespread throughout North America. It is a very fatal affection and on many farms it makes the raising of turkeys a difficult problem. The disease is characterized by thickening and ulceration of the cæca, and by extensive necrosis of the liver *Entamœba meleagridis* is a small amœba measuring about 8μ to 10μ in diameter. Turkeys probably become infected with this parasite by swallowing its encysted forms; young turkeys may possibly become infected from encysted amœbæ, which adhere to the shells from which they were hatched.

There has been no efficient treatment devised for the disease, since it is usually not noted until far advanced, but it can be avoided through keeping healthy stock on land which has never been infected by droppings from infected birds, and by carefully wiping eggs intended for hatching with formalin.

FLAGELLATA (Cohn emend. Bütschli)

The herpetomonads and the trypanosomes are the most important of the parasitic flagellates.

LEISHMANIA (Ross, 1903)

The three parasites belonging to this genus which require mention are included by some authorities in the genus *Herpetomonas* but the differences with respect to habit of life justify the recognition of a distinct genus. Herpetomonads live in the alimentary tract of various insects, for example, of the common blow fly and are extracellular parasites. Their bodies in general are rigid. *Leishmania* is, on the other hand, an intracellular parasite and in the flagellated phase of its development its body is plastic and bends during locomotion. Three species are recognized in association with three distinct types of disease in man. It is probable that all of the Leishmaniases will be found, eventually, to be caused by a stage in the life cycle of insect-borne herpetomonads.

KALA AZAR

Leishmania donovani—Laveran and Mesnil, 1903

This disease occurs in certain parts of Asia. It was first noted in Assam, Northern India.

It is caused by *L. donovani* (Fig. 184). The parasite is rarely found in the blood; when it is seen there, it almost never occurs free but is found in variable numbers within phagocytic cells. It is, usually, easily found by an examination of the juice obtained from the spleen or lymph nodes by puncture with the needle of a syringe. The liver is enlarged and it, also, contains parasites. As the organisms are seen in preparations of spleen juice, they are small ovals measuring about 2μ in length and 1.5μ in width. They consist of cytoplasm, in which lie two chromatic bodies, one of them large and rounded, the other small and rod-like. This form of the parasite may multiply in the body of the host, by binary or multiple division.

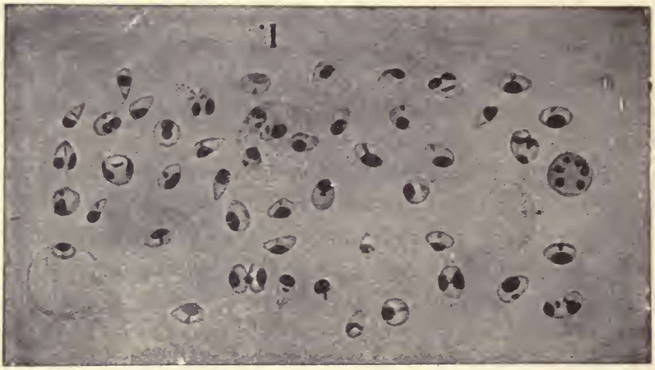


FIG. 184.—*Leishmania donovani.* Free organisms and several within cells. (*After Donovan, from Doflein.*)

If spleen pulp, or blood, containing such organisms be placed on a suitable culture medium, they will develop in three or four days, into herpetomonad forms. The large nucleus becomes the trophonucleus of the flagellate form, while the smaller, rod-like mass becomes the kinetonucleus, from which arises the flagellum. The method by which the infection is acquired is unknown; it is probably by the bite of an insect, perhaps a bedbug.

Kala azar is a chronic disease characterized by emaciation, by an irregular fever and by considerable enlargement of the spleen. There is great loss in strength and energy.

Although there may be periods of apparent amelioration, the disease usually progresses steadily, in spite of treatment, to a fatal termination.

INFANTILE KALA AZAR

Leishmania infantum—Nicolle, 1908

Most authorities recognize the generalized leishmaniasis which occurs in various countries bordering on the Mediterranean as a distinct disease. Nevertheless, it is not certain that *Leishmania infantum* and *L. donovani* are not identical. It is confined almost wholly to young children in whom it usually runs a fatal course. This disease has been transmitted to lower animals and the infection occurs naturally in dogs, especially those of infested districts. Recent investigations indicate that the dog furnishes a source of infection for human beings and that transmission is affected through the agency of a species of flea.

LOCALIZED LEISHMANIASIS (DELHI BOIL)

Leishmania tropica—Wright, 1903

The localized forms of leishmaniasis occur in widely distributed localities throughout the tropics, and numerous local names have been applied, Aleppo Boil, Oriental sore, Bagdad Boil, Biskra Button, etc. In South America likewise a number of local names have been applied, Espundia, Uta, Bubas, Braziliana and Forest Yaws. In certain forms of the disease the mucous membranes are invaded with loss of tissue of the nose and palate causing great deformity.

The parasites are found at the spreading edge of the lesions. As they occur in the ulcer they are oval parasites, almost identical with those which are found in the spleen of persons suffering from kala azar. If infected material be placed on a culture medium, flagellated forms develop. In many cases the organisms are difficult to find.

Delhi boil is a painless ulcer, covered by a dry scab. It usually occurs about the face, or other uncovered portions of the body. If the sore be left untreated, it cures itself after some months. In countries where it occurs, Delhi boil is particularly liable to form at the site of a cut or abrasion. It is possible that, in some cases, the infection may be carried to a wound by house flies.

The condition may be treated by free excision although it runs a self-limited course. In places where it is endemic, care should be taken to avoid the possibility of infection by carefully protecting all wounds, no matter how small.

TRYPANOSOMA (Gruby, 1843)

Trypanosomes are parasitic in insects, fish, reptiles, birds, and mammals in all parts of the world. Many of them seem to be harmless parasites; others cause very serious diseases.

Sleeping sickness, since it affects human beings, is regarded as the most important of the diseases due to trypanosomes.

SLEEPING SICKNESS

Trypanosoma gambiense—Dutton, 1902

Sleeping sickness is a disease of man caused by *Trypanosoma gambiense;* it is usually transmitted by the bites of *Glossina palpalis*, a tsetse fly. It is probably transmitted by all tsetse flies.

In South Central Africa, a number of men have been infected with trypanosomes which differ from *Trypanosoma gambiense.* The most important of them is *Trypanosoma rhodesiense;* the others are identical with trypanosomes usually found in animals. *Trypanosoma rhodesiense* causes a rapidly fatal disease uninfluenced by any treatment. The disease caused by *Trypanosoma rhodesiense* has not been observed in epidemic form. *Trypanosoma rhodesiense* is usually transmitted by a tsetse fly of another species, *Glossina morsitans.* In morphology it differs from *Trypanosoma gambiense* in that, in the blood of experimental animals, forms occur in which the trophonucleus is posterior to the kinetonucleus.

Sleeping sickness occurs only in those parts of Africa where tsetse flies exist.

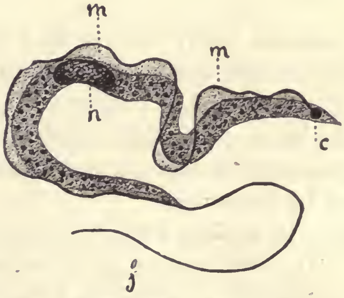


FIG. 185.—*Trypanosoma granulosum.* *n*, Tropho-nucleus; *m*, undulating membrane; *c*, kinetonucleus; *f*, flagellum. × 2000 diam. (*After Laveran and Mesnil from Doflein.*)

Trypanosoma gambiense (Fig. 186) is somewhat fusiform in shape and measures about 17μ to 25μ from the posterior extremity to the tip of its flagellum. A large tropho-nucleus is situated near the center of the trypanosome; a smaller, kinetonucleus lies near its posterior end. From this smaller nucleus a filament arises, which runs the whole length of the parasite and extends from its anterior end as a free flagellum. Where the filament runs along the body, the periplast extends over it to form the undulating membrane. The trypanosome moves by means of the undulating membrane and flagellum and also through the contraction of the myonemes which lie in the ectoplasm. In the blood, *Trypanosoma gambiense* multiplies by binary division. It is not impossible that it may multiply in other ways, as do other trypanosomes; for example, a trypanosome of frogs loses its locomotory apparatus and forms a sphere, then the sphere divides into many small spheres, each of which becomes a trypanosome. Sometimes *Trypanosoma gambiense* loses its locomotory apparatus and forms a sphere; these forms are found in the organs of infected animals. They are probably more resistant, resting forms and a single trypanosome may be formed from some of them.

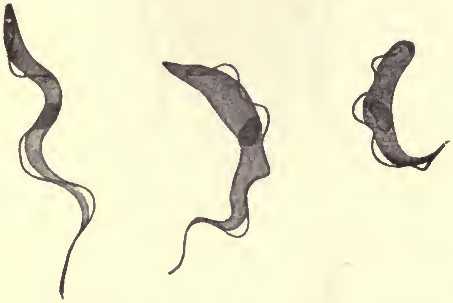


FIG. 186.—*Trypanosoma gambiense.* (*After Minchin, from Doflein.*)

Trypanosomiasis is easily transmitted to susceptible animals by inoculation. It is possible that the disease may be transmitted occasionally, in this way, by the mere mechanical exchange of infected material, through an insect's bite, from an infected to a healthy individual. But, as a rule, the disease can only be transmitted by the bites of *Glossina palpalis* in which the organism has developed (Fig. 187); the fly is not usually infective until three weeks after it has fed on an infected person, and it retains its infecting power for some months.

An incubation period of at least ten days intervenes between the bite and the appearance of symptoms, but this period may be much longer, for trypanosomiasis may manifest itself in apparently healthy negroes several years after they have left localities in which the disease could have been acquired. The disease sometimes causes death within three or four months; but it may last for one or more years. It is a chronic, wasting affection, characterized by loss of strength and energy, and by an irregular fever. A change in the mentality, red blotches on the skin, and enlargement of the lymphatic glands, are all early signs of the disease. In the later stages. beadache,

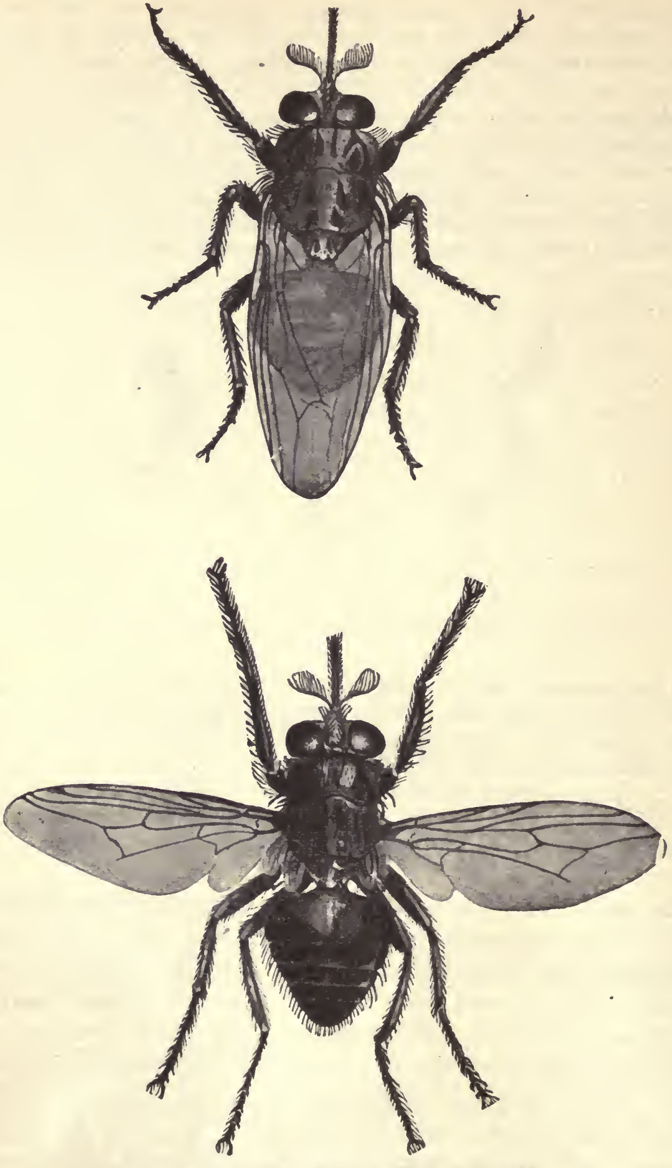


Fig. 187.—*Glossina palpalis.* (*After Doflein.*)

mania, uncontrollable sleep, and other nervous symptoms may be present. Death rarely results from trypanosomiasis alone; the patients usually succumb to one of the secondary infections, to which their reduced condition makes them especially liable. The symptoms are due to damage done both by the mechanical presence of the parasites and to a trypanotoxin produced by them. The parasites not only live in the blood and other fluids of the body but are found in the tissues of various organs. They are distributed throughout the tissue of the brain and their presence is associated with infiltration of the peri-vascular lymph spaces with large numbers of lymphocytes.

The recognition of trypanosomiasis depends upon the demonstration of the parasites. They may be found in fresh or stained preparations of the blood, in the juice obtained by aspirating an enlarged lymphatic gland, or in the cerebrospinal fluid. The examination of the blood is the simplest method of searching for trypanosomes; the examination of gland juice is the most efficient one.

The improvement in the methods of treating trypanosomiasis during the past ten years (1901-1911) affords an excellent example of the value of laboratory work. Before 1901 arsenic, given in some inorganic form, was the only drug known to have any effect on trypanosomiasis. Inorganic arsenic drives the parasites from the blood and improves the patient's condition. Unfortunately, the trypanosomes usually reappear and, then, they have become resistant to arsenic so that the patient succumbs in spite of repeated doses. Many organic compounds of arsenic were experimented with in the hope of finding an efficient trypanocide and several valuable drugs have been found: "Atoxyl" which is the sodium salt of para-amido-phenyl-arsenic acid, acetylated atoxyl, and arsenophenylglycin, are all organic compounds of arsenic. They are much more effective than is arsenic itself. Similar organic compounds of antimony and tartar emetic are as effective, while certain aniline dyes have a distinct trypanocidal value. It has been found that trypanosomes may become resistant to any one of these drugs, and that drugs may destroy some stages of the trypanosome while they are unable to destroy others. In order to give the parasites no opportunity of acquiring resistance to any drug, and in order to destroy them at all stages of their development, the following general rules are now observed in the treatment of trypanosomiasis. The drugs employed should be alternated, and they should be given as early in the disease as possible, and in as large doses as possible. It is probable that these principles will be found to be of value in the treatment of other diseases caused by protozoa.

The prevention of the disease depends upon the avoidance of the water's edge, where *Glossina palpalis* exists, and of the proximity of persons or animals infected by trypanosomiasis. The most usually successful way of recognizing infected persons is by the discovery of trypanosomes in the fluid aspirated from their enlarged lymphatic glands. By experimental inoculation, and by the examination of animals naturally infected, it has been shown that wild and domestic animals of many species may be infected with the trypanosomes which are usually the cause of sleeping sickness. Some animals are killed by the infection. All of the larger animals resist the infection for very considerable periods and it is possible that many of them are tolerant to the infection. This is especially true of the larger ruminants. Therefore, antelope, buffalo—game—should be driven away or destroyed in the neighborhood of human habitation in order to remove a dangerous reservoir of infection.

Human Trypanosomiasis of South America

Trypanosoma cruzi—Chagas, 1909

This disease is caused by *Trypanosoma cruzi* (*Schizotrypanum cruzi*) and is transmitted by the bites of a reduviid insect, *Lamus megistus*. It has been found only in Brazil.

Trypanosoma cruzi may be either free in the blood plasma or lie within a red cell. It multiplies, in the tissue cells of muscles and organs, by losing its locomotory apparatus and forming Leishmania-like bodies which multiply by repeated divisions and develop into new trypanosomes. These young parasites leave the destroyed tissue cell where they were produced and enter the blood vessels.

The disease is a chronic one, characterized by irregular temperature, by wasting, œdema, and enlargement of the spleen and lymphatic glands. It occurs chiefly in young children and is often fatal. It may be prevented by avoiding the insect which transmits it—the habits of *Lamus* resemble those of a bed bug.

TRYPANOSOMIASES OF ANIMALS

Several diseases, of great economic importance, which affect domestic animals, are caused by trypanosomes. The following are the most important. Tsetse-fly disease, or nagana, of Southern Africa, is caused by *Trypanosoma brucei* (Plimmer and Bradford) and it is transmitted by the tsetse fly, *Glossina morsitans;* it affects all domestic animals.

In South America, mal de caderas, a disease of horses, is caused by *Trypanosoma equinum* (Voges); it is probably transmitted by a biting fly, *Stomoxys.*

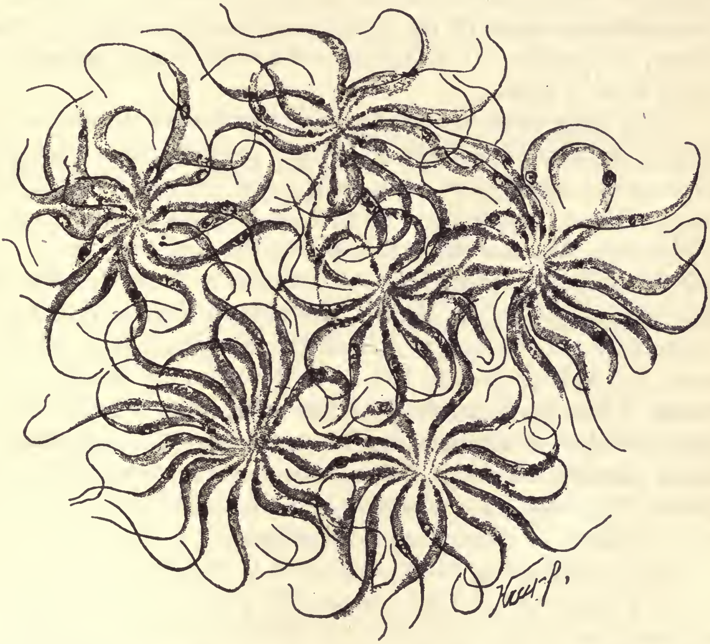


FIG. 188.—Colonization in *Trypanosoma lewisi* (Kent). (*From Doflein.*)

All through Asia, surra, caused by *Trypanosoma evansi* (Steel), is a severe disease of cattle and equines; it is probably transmitted by biting flies.

Trypanosoma dimorphon (Laveran and Mesnil) and many other trypanosomes, more or less closely allied to it, cause diseases of horses, cattle, and other domestic animals in many parts of Africa; they are probably all transmitted by the bites of flies.

One of the commonest trypanosomes is *Trypanosoma lewisi* (Kent). It is usually a harmless parasite and it is found in rats in all parts of the world. It is transmitted through the rat flea. Trypanosomes ingested by fleas develop and are excreted in a resting stage with the fleas' droppings. Rats, swallowing the infected droppings, or fleas, become infected. It is not transmissible to other mammals.

Dourine or maladie de coit, is a serious disease of equines; it is caused by *Trypanosoma equiperdum* (Doflein). This disease was brought to North America by an imported Percheron stallion. It is now endemic in some of the western states and in part of southern Alberta, in Canada. It is transmitted by coitus and, perhaps, rarely by the bites of fleas.

A very large trypanosome, *Trypanosoma theileri* (Bruce), occurs in cattle in southern Europe and in Africa. A similar large trypanosome, *Trypanosoma americanum*, has been found in cattle in the United States. These trypanosomes seem to do no harm to their hosts.

Although there are slight differences, the symptoms are much the same in all the trypanosomiases of animals, and they much resemble those which occur in the diseases produced in men by trypanosomes. Occasionally, as in nagana, an animal trypanosomiasis may run an acute course, and kill the host in two or three weeks, but usually, they are diseases of long duration, characterized by irregular fever, œdemas and progressive loss of strength, weight, and energy. Localized areas of œdema beneath the skin and about the genitals are especially seen in dourine; *Trypanosoma equiperdum* is most easily found by examining serum obtained by puncturing these œdemas.

The remaining flagellates, mentioned in the classification on page 14, are unimportant. They are usually parasites of the urinary or intestinal tracts and they may be associated with inflammation of these parts.

SPOROZOA (Leuckart, 1879)

This class contains many very important pathogenic parasites.

COCCIDIA (Leuckart)

Coccidia of various species are parasitic in the epithelial cells lining the intestines of mice, horses, cattle, pigs, goats, and other animals.

In Europe, *Eimeria stiedæ* (*Coccidium cuniculi*) sometimes causes an enteritis of cattle; in East Africa, a coccidium causes a serious disease of cattle. Other coccidia kill many young pigeons, grouse, and chickens. Coccidia have been found in the liver and intestinal tract of man.

COCCIDIOSIS OF RABBITS

Eimeria stiedæ—Lindemann, 1865

Syn.: *Coccidium cuniculi*

The coccidium causing this disease is the best known of the coccidia infecting mammals.

This coccidium is parasitic within the epithelial cells of the intestine and within the epithelium lining the bile ducts. Adult, asexual forms measure from 20μ to 50μ in diameter and they produce from 30 to 200 merozoites. The merozoites infect other epithelial cells where they may again multiply asexually or they may develop into male and female forms destined to multiply sexually. One of the microgametes, produced by a microgametocyte, fertilizes a macrogamete and an oöcyst is developed. Within the oöcyst a number of sporoblasts form, which contain two spores each. The oöcysts are passed with the fæces and if they are ingested by a suitable host the spores are set free, when the cyst reaches the intestine, and a new infection is commenced.

Since the cells parasitized by the coccidia are destroyed, it is evident that a severe infection may do a great deal of harm and interfere with the functions of both intestine and liver. The disease may be limited by making it impossible for uninfected animals to come into contact with the droppings of infected stock.

AVIAN COCCIDIOSIS

Coccidium infection is of frequent occurrence among birds, and especially those of domestic varieties, without causing serious symptoms. It is known, however, to cause severe epidemics in certain species, and when present in milder form should be regarded as antagonistic to health. *Entamœba meleagridis* the organism of "Black head" in turkeys, from its peculiar relationship to the tissues, has been erroneously regarded as a form of coccidium.

HÆMOSPORIDIA (Danilewsky emend. Schaudinn)

The most important parasites of this order are those, belonging to the Genus *Plasmodium*, which cause malaria in man. Organisms similar to these are parasitic in the red blood cells of apes, bats and antelopes. *Proteosoma* and *Hæmoproteus* are two genera parasitic in the red blood cells of birds. It was the study of these avian parasites which led to the discovery of the way in which malaria is transmitted by the bite of the mosquito.

PLASMODIUM (Marchiafava and Celli, 1885)

At least three species of this genus are parasitic in man: *Plasmodium vivax* (Grassi and Feletti), the cause of tertian malaria, *Plasmodium malariæ* (Laveran), causing quartan malaria, and *Plasmodium falciparum* (Welsh), which causes aestivo-autumnal malarial fever.

MALARIA

Malaria is a disease caused by an amœboid parasite of the red blood corpuscles. It is transmitted by the bite of anopheline mosquitoes in which the parasite has completed the sexual cycle of its development. It exists in all parts of the tropical and subtropical world (Fig. 189).

A young malarial parasite or sporozoit, derived from the mosquito enters a red cell and supports itself by living upon the cell's substance. The parasite grows, proceeds to multiply asexually and divides into a number of merozoites which are set free by the rupture of the red cell. Those of the merozoites which escape ingestion by the white cells of the blood enter red cells where they may again multiply asexually, or they may develop into sexual forms. When blood, containing malarial parasites, is ingested by a suitable mosquito, all the parasites, except the adult sexual ones, are digested and die. Soon after they are ingested, the macrogametocyte extrudes polar bodies and becomes a macrogamete and the microgametocyte produces several microgametes, one of which enters and fertilizes the macrogamete. Through the fusion of macrogamete and microgamete a copula is formed, which since it is motile is called an oökinet. This makes its way until it comes to lie just beneath the outer surface of the mosquito's stomach. There it develops, as an oöcyst, until it reaches several times its original size. It divides into a number of areas, or sporoblasts, each of which subdivides to form many very small, hair-like sporozoites. When the oöcyst bursts, some of the sporozoites pass forward to find their way into the salivary glands of the mosquito, and, when it bites, they are extruded, with the saliva, into the

DESCRIPTION OF PLATE I.

(A diagram reproduced from Greene's Medical Diagnosis)

Plasmodia of three varieties. Stained by Wright's stain.

In this plate the chromatin of the parasites is shown in red while the pigment granules appear as black dots.

THE QUARTAN PARASITE (*P. malariæ*)

1–9 Asexual multiplication. 10. Adult gametocyte. 11. Normal red cell. 12. Flagellating microgametocyte.

THE TERTIAN PARASITE (*P. vivax*)

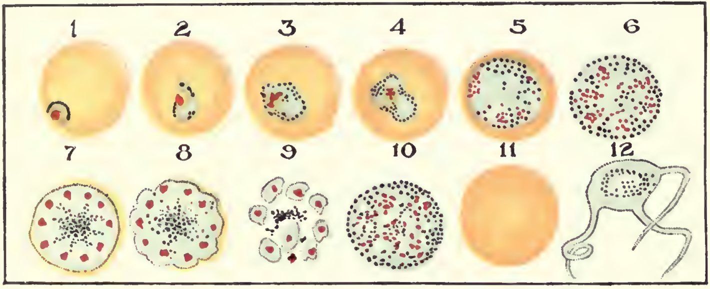
13–21. Asexual multiplication. 22. Flagellating microgametocyte.

THE ÆSTIVO-AUTUMNAL PARASITE (*P. falciparum*)

23–31. Asexual multiplication; 25 and 26 are doubly infected cells.
32–35. Gametocytes. 36, 37. Flagellating microgametocyte.

PLATE I.

THE QUARTAN PARASITE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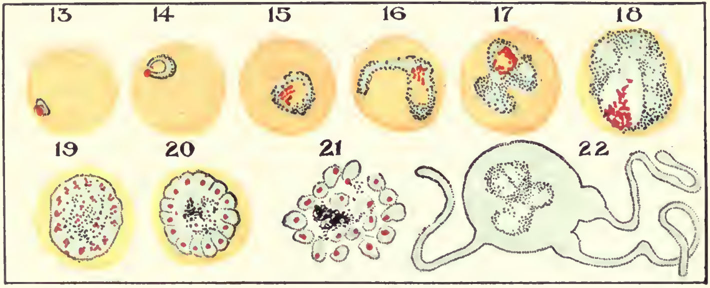
THE TERTIAN PARASITE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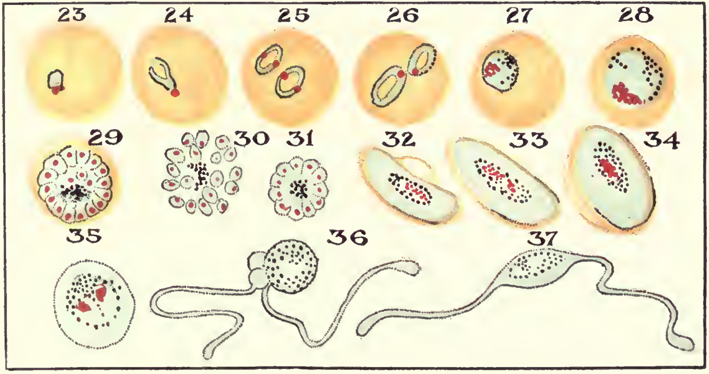
THE ÆSTIVO-AUTUMNAL PARASITE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body of the person from whom blood is being sucked. The entry of a sporozoite into a red cell recommences the cycle of development which has just been described. If the adult sexual parasites are not taken up by a mosquito they die off in the blood, but some of the female forms may live for years and then divide parthenogenetically, without a precedent fertilization, to produce several young parasites. It has been suggested but never demonstrated that the sporozoites may enter eggs lying in the ovaries of infected mosquitoes and that mosquitoes, hatched from such eggs, may inherit the infection from their parent and that they, also, are able to transmit malaria.

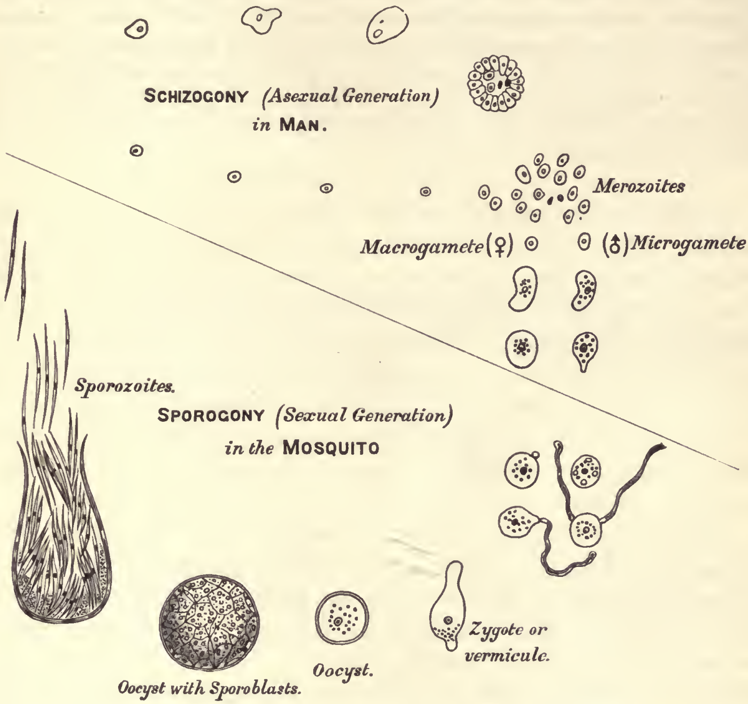
SCHIZOGONY *(Asexual Generation)*
in MAN.
Merozoites
Macrogamete (♀)
(♂) *Microgamete*
Sporozoites.
SPOROGONY *(Sexual Generation)*
in the MOSQUITO
Zygote or vermicule.
Oocyst.
Oocyst with Sporoblasts.

FIG. 189.—Diagram illustrating the human and mosquito cycles of existence of the malaria parasite. (*After Martin's General Pathology.*)

In fresh preparations of blood, a malarial parasite is seen as a body of varying size, which is more refractile and of a lighter color, than the red cell which contains it. In its growing phase it has distinct amœboid movement and the pigment granules lying in it are in active motion. In preparations, stained by a modification of Romanowsky's method, every malarial parasite is seen to possess a definite purple nucleus surrounded by blue-staining cytoplasm. Young parasites measure less than a fifth of the diameter of a red cell in width; adult parasites may completely fill the cell which contains them. Malarial pigment is the waste product which results from the digestion of the hæmoglobin of the red cells by a malarial parasite, and consequently, since they have digested more hæmoglobin, the older parasites contain more pigment than do the younger ones. A mature asexual parasite finally segments into a number of merozoites; *Plasmodium vivax* forms about eighteen, *Plasmodium malariæ* about eight merozoites. The adult sexual forms of *Plasmodium falciparum* are shaped like a crescent, and for that reason it is described by some as the type of a genus *Laverania*, under the name of *Laverania malariæ*. The three malarial parasites of man may be distinguished from one another by these peculiarities as well as by other, lesser differences in themselves and in the red cells which they parasitize.

When a mature, asexual, malarial parasite bursts, it sets free young parasites and a toxin. Practically all of the parasites, present in a person suffering from typical acute malaria, mature and burst at the same time and the considerable amount of toxin, set free in this way, produces a paroxysm characterized by chills and fever. The parasites of *Plasmodium vivax* mature in forty-eight hours. Consequently, a person infected by it has a chill when schizogony occurs, on every third day, and the disease caused by it is called a *tertian fever*. *Plasmodium malariæ* matures in seventy-two hours, causes an attack of ague on every fourth day and the disease produced is called *quartan fever*. Patients infected by *Plasmodium falciparum* often have a *quotidian fever* with a daily rise in the temperature, although a three day period may be recognized in some cases. There are three stages in the paroxysm: during the chill, the patient feels cold; in the hot stage he feels warm—his temperature is above normal during both stages; in the sweating stage the temperature falls to normal and the patient's discomfort becomes much less.

The regularly recurring chills and fever constitute the only symptoms characteristic of malaria and a regular rise in temperature on the third or fourth days of an illness is strongly suggestive of a malarial infection. The type of disease and the symptoms, produced by a malarial infection, may vary almost indefinitely according to the precise way in which the host is harmed by the infection. Consequently, an enumeration of the clinical manifestations of malaria is of less importance to a student than is an understanding of the way in which the malarial parasites harm their host. The malarial parasites destroy the red cells and thus cause an anæmia with the symptoms which result from it. Secondly, they produce toxins which may cause both acute and chronic intoxications. The acute intoxications are seen in the elevation of body temperature and in unconsciousness in some pernicious forms of malaria; malarial neuritis is an example of chronic intoxication. Lastly, malarial parasites may do harm by blocking the capillaries and causing the death of the cells which are cut off from the circulation; the symptoms which result depend upon the functions of the cells which are destroyed. If the disease be long continued, with a high temperature, the degenerative changes which usually result from chronic disease and constant fever are produced in the patient.

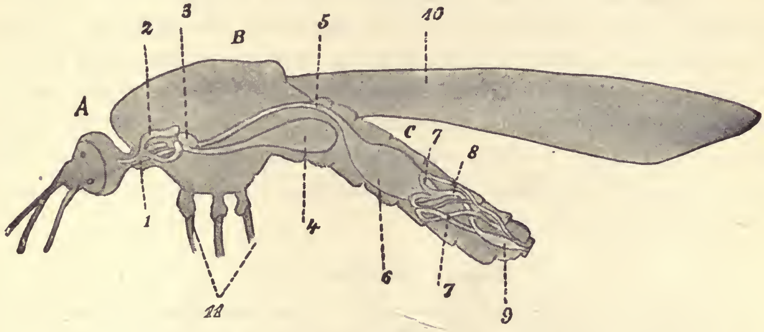

FIG. 190.—Longitudinal section of *Anopheles*. *A*, head; *B*, thorax; *C*, abdomen; 1, œsophagus; 2, salivary glands; 3, dorsal reservoir; 4, ventral reservoir; 5, canal entering stomach; 6, stomach; 7, malpighian tubes; 8, hind-gut; 9, rectum; 10, wings; 11, legs. (*After Grassi, from Lang and Doflein.*)

The definite diagnosis of malarial fever depends upon the demonstration, in a patient, of the malarial parasite, or of the pigment produced by it.

Quinine has a specific action on the malarial parasite and is the most valuable drug available for the treatment of the disease. It must be given promptly in full doses. Treatment must be continued until all parasites disappear from the blood.

Malaria, since it is a disease which is caused by a parasite and transmitted by an insect, may be prevented by measures directed either against the parasite or against the transmitting agent. Malaria is caused by a *Plasmodium* and transmitted by the bites of mosquitoes belonging to the *Anophelinæ*. The disease may be combated by destroying the parasite, in infected persons with quinine, and by isolating such persons under mosquito nets so that mosquitoes may never have an opportunity of ingesting the parasites which they harbor in their blood. Malaria may also be prevented by destroying the mosquitoes which transmit it. The most efficient way of getting rid of mosquitoes is to make it impossible for them to breed. The eggs of a mosquito are laid in water, and water is absolutely necessary for the larval and pupal stages, which must be passed through before the adult mosquito is produced. Fish destroy developing mosquitoes and large sheets of water are too rough for them—so mosquitoes must have, for breeding, rather small collections of fresh water free from fish. Mosquitoes will soon disappear from a locality if all such collections of water, within a quarter of a mile of it, are filled up, drained, or covered with a film of coal oil so as to make it impossible for the mosquitoes to breed in them. Those who live in a malarious district should protect themselves from mosquito bites by the careful use of mosquito-netting. By the simple observance of these evident indications, malaria has already been banished from several localities in which it was formerly endemic.

BABESIA (Starcovici, 1893)

This order is often called PIROPLASMA. It includes many parasites, which cause diseases of considerable economic importance in horses, cattle, sheep, and dogs. One of the best-known species is *Babesia bigemina*, which causes Red-Water or Texas Fever of cattle. The parasites which are associated with the numerous babesiases are distinguished from one another by the host in which they are found, by slight differences in their morphology and by their inoculability into various animals.

RED WATER

Babesia bigemina—Smith and Kilborne, 1893

Red water is one of the names given to a disease of cattle which is characterized by hæmoglobinuria; in the United States it is often called Texas cattle fever. It is caused by *Babesia bovis* (*bigemina*) (Fig. 191). The parasite is transmitted by the bites of ticks, in North America, by *Boophilus annulatus*.

Red water occurs not only in the southern portion of the United States but almost everywhere in the tropics and in many of the warmer parts of the temperate zones.

The parasite is a pear-shaped organism which usually lies within a red cell. It measures from 2μ to 4μ in length and about 1μ in breadth. In fresh preparations they appear as refractile bodies possessed of slight amœboid movement; in stained preparations they are seen to consist of a blue-staining cytoplasm which contains a mass of chromatin at its broader end. Multiplication is accomplished by simple division into two or more parts; it is possible that schizogony and sporogony may also occur. The parasites are often very scarce in the peripheral circulation but are much more numerous in the organs and particularly in the spleen. The disease can be transmitted, experimentally, from bovine to bovine by the inoculation of blood which contains parasites; normally, it is transferred from animal to animal by the bites of a tick. The species of tick which carries red water is not the same in all parts of the world.

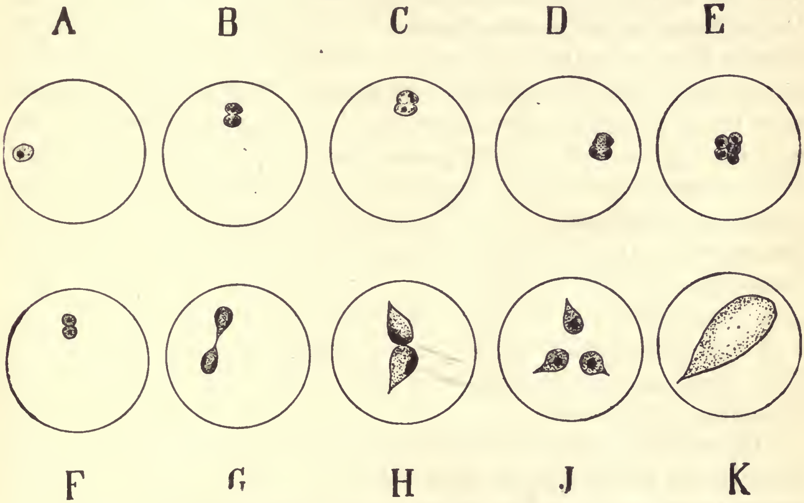

FIG. 191.—*Babesia bigemina.* Various stages of development in red blood cells. *A*, young parasite; *B*, a twin-form; *C-E*, a multiple division; *F-K*, large pear-shaped forms. (*After Doflein.*)

Ten days intervene between the bite of the infecting tick and the first sign of the infection. The temperature rises, it may be, to 106°, or more, and it remains high for a week. The animal is evidently very ill, it has no appetite, and it rapidly loses strength and weight. Many red cells are destroyed and anæmia may be marked. The urine is albuminous and it is red because of the hæmoglobin which it contains. Death may occur in very acute cases as early as the second day. Animals which recover from a severe attack are usually immune to the disease. The immunity is not an absolute one, however, for blood taken from such recovered animals is often infective; the parasite probably exists in them in a latent form through the establishment of a tolerance on the part of the host.

There is no specific treatment for babesiasis. Some of the aniline drugs, used in the treatment of trypanosomiasis, such as trypan-blue, are of some value.

Many districts are kept free from red water by not allowing cattle coming from infected districts to enter them. Where it exists, the disease is controlled by destroying the ticks on cattle with poisonous washes and by occasionally plowing, or burning over, the pastures in order to destroy ticks which have dropped to the ground. In the United States, cattle on some farms are kept free from ticks, and consequently from red water, by a manœuvre which takes advantage of the way in which the tick transmits the disease. The adult tick remains upon her host until she is ready to deposit her eggs; she then drops off, lays her eggs and dies. The young ticks, hatched from these eggs, attach themselves to new hosts and it is through their bites that the disease is transmitted. Therefore, since the disease is transmitted by the progeny of ticks which have fed upon infected mammals, susceptible cattle may be protected from the disease by preventing young ticks from reaching them. This may be done by not allowing them to feed over fields where ticks may have been dropped until sufficient time, about ten months, has elapsed for all the ticks and their progeny to have died of starvation.

There are a number of parasites which although closely related to the Babesias are usually placed in other genera. Most of these have been shown to be transmitted by ticks of various species.

EAST COAST FEVER

Babesia parva (Theiler, 1903)

Syn.: *Theileria parva*

This parasite also is found in the red blood corpuscles of cattle. It is the cause of a disease which is characterized by severe anæmia. The intracorpuscular forms vary in form, some being slender and rod-shaped, the others being more rounded or pear-shaped. They may be arranged to form a cross, and this is not due to segmentation but to fortuitous grouping in heavily infected cells. They are regarded as gametocytes, for although such blood is infectious for ticks it will not produce infection when injected into normal cattle. The multiplicative or asexual phase of the organism is restricted to certain organs, especially the lymph nodes, spleen and bone marrow. The tissue from these organs when injected into normal cattle produces infection.

OROYA FEVER

Bartonella bacilliformis—Strong, Tyzzer and Sellards, 1915

A human disease, characterized by rapidly developing and severe anæmia associated with an irregular fever, occurs in certain mountain valleys in Peru. The red blood corpuscles harbor slender rod-shaped and small rounded organisms in numbers varying with the severity of the disease. The rods are frequently arranged in chains of two, three or even four, and present deeply stained granules at one or both extremities. Examined in fresh preparations they are found to move slowly without marked change of shape through the interior of the red cell. Cross-forms are rare and probably represent fortuitous arrangement rather than segmentation of the organisms. The endothelium of the blood-vessels of the lymph nodes, spleen and liver contain organisms in various stages of development, small rod-shaped forms similar to those of the red cells eventually being formed. The distention of the endothelial cells is often sufficient to occlude many of the blood-vessels and in the lymph follicles of the large intestine this has apparently led to the necrosis of the surrounding tissue and ulceration. The organism of this disease is smaller than that of East Coast fever, the rod forms being very slender and nuclear material is not so readily differentiated. Its resemblance in other respects, together with the similarity of its distribution in the tissues, indicates a relationship to this group of organisms.

The disease has not been transmitted to lower animals. Carrion, a Peruvian student, who inoculated himself with the blood of a patient suffering from Verruga peruviana died from a disease which may have been Oroya fever, although the evidence on this point is inconclusive. Oroya fever and Verruga peruviana not infrequently occur simultaneously in the same individual, just as the latter disease is frequently complicated by malaria, and this together with the result of Carrion's experiment led many Peruvian physicians to the erroneous belief that

Oroya fever and Verruga were different stages or manifestations of a single infection. Verruga is, however, readily transmitted to lower animals. The mode of transmission of Oroya fever has not been conclusively determined.

Anaplasmosis

In a pernicious anæmia of cattle, and at times in babesiasis, the red blood corpuscles contain minute, deeply stained, rounded bodies. These are frequently in pairs and are commonly situated near the margin of the cell so that they have been given the name *Anaplasma marginale* (Theiler), by those who are convinced of their parasitic nature. They have also been found in other domestic animals and similar structures are found normally in all individuals of certain species, as for example, the mouse. Since the bodies of this general appearance which occur in normal animals are evidently to be regarded as nuclear material certain investigators are inclined to doubt the parasitic nature of *Anaplasma.*

Sarcosporidia (Balbiani)

Different species of this order are frequent parasites of all the domestic animals, of mice and, occasionally, of man. Mice are killed by them and it is possible that they may produce ill effects in men and domestic animals but no definite disease is associated with their presence. Though they may occur in any part of the body, they are most numerous in certain muscles, such as those of the larynx and œsophagus, which are near the alimentary canal. For this reason it seems possible that they may enter the bodies of their hosts with food, but our knowledge of their life history is incomplete.

Haplosporidia (Caullery and Mesnil)

One unimportant parasite, *Rhinosporidium kinealyi,* belonging to this order is parasitic in man. It has been found in small tumors of the nose and external ear. A few cases have been reported from Asia and from North and South America. In the tumors cysts occur which are filled with spores.

Myxosporidia (Bütschli)

There are many species in this group which are parasitic in fishes and certain arthropods but not in higher animals. The classification is based largely on the character of the spores produced. The latter are provided with a resistant membrane or shell and with polar capsules, each of which contains a coiled filament which when extruded serves to anchor the spore. The spores are produced continuously within the protoplasm of the mother organism which may be situated in any part of the body of the host. Their presence may, in severe infections, cause boil-like lesions. Epizoötics, killing enormous numbers of fish, are sometimes caused by these parasites.

Microsporidia (Balbiani)

Protozoa belonging to this order do not produce disease in man. They are the cause of a disease of bees, and they are of particular interest because one of them, *Nosema bombycis*, causes Pébrine, a serious disease affecting silk-worms (page 937).

Infusoria (Leddermüller, 1763)

Most of the parasitic infusoria occur in the alimentary tracts of their hosts. Harmless infusoria are found in the stomachs of many herbivorous animals and also in the large intestine of the frog. *Balantidium coli* is a common and apparently innocuous parasite of the cæcum and large intestine of the pig, but it may cause a severe and fatal inflammation of the large intestine in man. One or two other infusoria occasionally produce similar symptoms in man. Other species of infusoria are parasitic on fish. Some of these are harmless, but some by finding their way into the gills or beneath the scales, cause serious diseases.

BALANTIDIUM ENTERITIS

Balantidium coli—Malmsten, 1857

Balantidium coli is the most important of the infusoria parasitic in man and may cause a form of dysentery.

This organism measures about 150μ in length and 50μ in breadth. It is covered with cilia; its cytoplasm is differentiated to form oral and anal areas and it contains digestive and contractile vacuoles. It multiplies by simple transverse division, either with or without a precedent conjugation. It may encyst, and this is the form in which the parasite is transmitted from one host to another.

High enemata of mild antiseptics have been used in the treatment of this infection.

PARASITES OF UNCERTAIN POSITION

In Panama, there is a disease of man, somewhat resembling one form of tubferculosis, which is caused by a parasite called *Histoplasma capsulatum.* The only known stage of this parasite greatly resembles the non-motile form of *Leishmania donovani;* but it contains only one, not two masses of chromatin. This organism was at first thought to be protozoön but is now considered to be a fungus.

The name *Toxoplasma* has been given to a group of organisms which usually inhabit the white blood cells of vertebrates. They do not produce pigment. They have been found in animals and birds of several species in many parts of the world. No parasite of this genus has been found in man.

CHLAMYDOZOA (Prowazek, 1907)

This name is given to certain bodies because their presence excites the cell containing them to produce a substance which surrounds them like a cloak. The exact nature of these bodies is disputed; it is even doubtful whether they are parasites, or whether they are merely the expression of some morbid change, produced in the cells, by an unseen virus which causes the disease. They have been found in trachoma, a disease of the eyelids of man, in hydrophobia, in *Molluscum contagiosum,* a skin disease, in smallpox, in vaccinia, and in scarlet fever. They are mentioned with the protozoa because, if they are parasites, they are probably more nearly allied to the protozoa than to the bacteria. They are extremely small bodies, some measuring only 0.25μ in diameter. They are spherical and occur within the cells. In preparations stained by Romanowsky's method they are colored like chromatin.

RICKETTSIA (Rocha-Lima)

Rickettsia is the name generally applied to a group of pleomorphic organisms which are associated with typus, Rocky Mountain fever and trench fever. These organisms vary in form from cocci to long threads of bacilliform organisms. They stain with difficulty and have not been cultivated. They are transmitted by insects. The organisms of trench fever and typus are carried by lice; that of Rocky Mountain fever is carried by a tick. (Wolbach, 1919, proved that the organisms causing Rocky Mountain Spotted Fever, which he calls *Dermacentroxenus rickettsi,* is carried by *Dermacentor venustus.*

ULTRAMICROSCOPIC VIRUSES (See page 119)

SPIROCHÆTA (Ehrenberg, 1833)

Many spirochætes are, apparently, harmless parasites in shell fish, in the alimentary canals of various animals and in the blood of fish, birds, and many mammals; other spirochætes produce disease in men and in lower animals.

Several spirochætes are parasitic in man. *Spirochæta dentium* and *Spirochæta buccalis* are harmless organisms which are found in tartar, about the teeth.

Spirochæta vincenti occurs in great numbers in a certain form of sore throat. Other spirochætes have been found in foul ulcers, and others have been found in association with bronchitis, urethritis and enteritis. All these are comparatively unimportant parasites. *Spirochæta recurrentis*, the cause of relapsing fever, is the most important of them.

RELAPSING FEVER

Spirochæta recurrentis—Lebert, 1874

The relapsing fevers may occur in any part of the world. They are caused by spirochætes and are transmitted by ticks and lice.

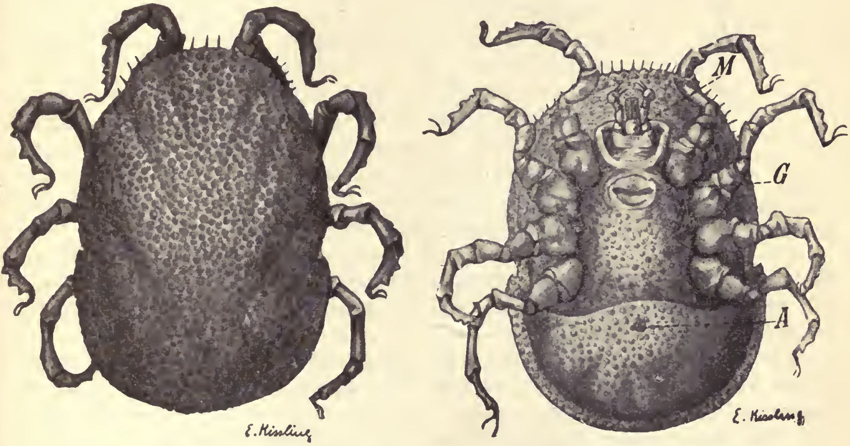

FIG. 192.—*Ornithodoros moubata.* (*Murray from Doflein.*)

The disease is carried by *Ornothodoros moubata* (Fig. 192) in Africa and wherever this tick exists. It is similarly carried in Persia and elsewhere by *Argas persicus*. Other ticks carry it in Mexico and in South America. In Europe, Asia and Northern Africa, the disease is usually transmitted by lice. There are grounds for believing that it may also be carried by other biting ectoparasites such as bedbugs.

The spirochætes causing relapsing fevers in man are sometimes described as belonging to different species mainly because experimental animals immune to infection by one of the spirochætes are susceptible to infection by another. This difference is not of specific importance since two strains can be developed from a single spirochæte neither of which affords immunity against infection by the other. Because of the similarity among these spirochætes in method of transmission and because of practical identity in the symptoms and treatment of the diseases which they produce, it is better to describe the relapsing fevers as one disease caused by *Spirochæta recurrentis.*

Spirochæta duttoni is a slender organism measuring from 14μ to 16μ in length; its thread-like body lies in a number of waves, which vary greatly in number, according to the way in which the preparation is made; consequently, the number of waves is not a constant character which can be relied upon for the identification of these spirochætes. This spirochæte is composed of an outer ectoplasmic sheath, and of an interior composed largely of chromatin; the sheath extends at either end into flagellum-like prolongations. Multiplication is usually accomplished by transverse binary division, sometimes by longitudinal division. Sometimes, perhaps most often toward the end of an attack of fever the spirochætes coil up tightly, within a cystlike matrix. Such encysted forms may lie within cells, *i.e.*, liver cells, and spleen cells; they are seen most frequently in the liver and spleen, and they are always present in the alimentary canal of ticks which have ingested spirochæte-infected blood. The chromatin of both free and encysted spirochætes may be fragmented, more or less regularly. In the tick, cysts, containing a spirochæte with fragmented chromatin, burst and set free the granules of chromatin. Some investigators believe that each granule develops into a spirochæte, others that this represents a degeneration and destruction of the parasite. It is not impossible that some such method of multiplication occurs in man.

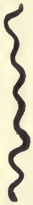

FIG. 193.—*Spirochæta duttoni.* (*After Doflein.*)

The form and exact way in which the spirochætes are transmitted is not completely known. Ticks can transmit the disease by their bites. Lice, which have become infected from feeding on patients, do so only when they are crushed and their bodies are rubbed into the wounded skin by scratching fingers. Similarly, infection may result when spirochætes contained in coxal fluid or fæces, dropped upon the skin by a feeding tick, find their way into the wound made by the tick's bite. It is probable that a tick, once infected, never loses its power to transmit the disease; the infection may be transmitted, from mother to daughter, through at least three generations of ticks.

An incubation period of about five days intervenes between infection and the appearance of symptoms. The fever is characteristic; it rises rapidly to, perhaps, 105° and it remains high for from three to five days. It then falls suddenly and there is no fever for from five days to two weeks. Then the temperature rises again. There may be from three to six such recurrences of fever before the illness ends. The definite periodicity of the relapses probably depends upon some more or less regular developmental change in the spirochætes since the latter are always most numerous in the blood during the height of the fever. The disease is not often fatal and "606" is a specific treatment for it. It can be prevented by avoiding lice and ticks.

TREPONEMA (Schaudinn, 1905)

Two species of this genus are very important parasites.

SYPHILIS

Treponema pallidum—Schaudinn, 1905

This disease, in all its diverse forms, is caused by *Treponema pallidum.*

The treponema is an exceedingly slender, thread-like organism, with a waved body which measures from 6μ to 14μ in length (Fig. 194). It greatly resembles the spirochætes, but differs from them in having each end drawn out to resemble a very slender flagellum. Very little is known of the life history of the treponema except that it multiplies by transverse division. It is transmitted by the contact of a lesion, containing the parasites, with the broken skin, or with a mucous membrane of an uninfected person.

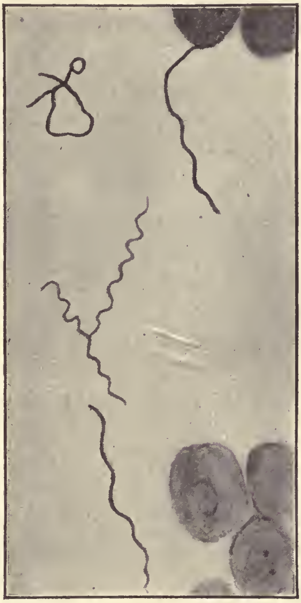

FIG. 194.—*Treponema pallidum* (in centre) and the *Spirochæta refringens.* (*Greene's Med. Diagnosis*)

Mercury and potassium iodide were formerly almost exclusively employed in treating syphilis. The search for an efficient drug for the treatment of trypanosomiasis has led to the discovery of other drugs which are of value in the treatment of syphilis, such as atoxyl (the sodium salt of para-amido-phenyl-arsenic acid) and its acetylated derivative, and of dichlorhydrate-diamido-arseno-benzol. The last-named drug, "salvarsan," "606," has proved of great importance in the treatment of syphilis.

YAWS OR FRAMBOŒSIA

Treponema pertenue—Castellani, 1905

This is a disease of the tropics which was formerly confused with syphilis. It is characterized by the presence, on any part of the body, of more or less numerous warty fungoid lesions which tend to ulcerate. In this disease a primary lesion appears after a period of incubation and this is followed after another interval by a general eruption. It is not a venereal disease as is the case with syphilis. It is caused by a slender spirochæte, *Treponema pertenue*, which is morphologically identical with the organism of syphilis. Animals which have been immunized to one of these diseases are found to be still susceptible to the other. The organisms in yaws are present in enormous numbers in the hypertrophied and swollen epidermis of the lesions whereas in syphilis they are confined to the deeper tissues. The disease responds more favorably even than syphilis to treatment with salvarsan.

OTHER SPIROCHÆTAL DISEASES

LEPTOSPIRA

Spirochætaform organisms are the cause of other diseases. *Leptospira icteroides* occurs in yellow fever. *Leptospira icterohæmorrhagiæ* is the cause of infectious jaundice. Spirochætes of a form similar to those sometimes found in apparently normal rats and mice occur in the blood of persons suffering from the fever which sometimes follows the bites of, especially, rats.

Spirochætes cause diseases of geese in southern Russia and of fowls in Brazil and in other tropical countries. The spirochæte of fowls, *Spirochæta gallinarum*, P. Blanchard, is transmitted by ticks, *Argas persicus;* the means by which the goose spirochæte, *Spirochæta anserina*, Sacharoff, is carried is not known.

DIVISION IX

MICROBIAL DISEASES OF INSECTS

INTRODUCTION*

Microbial diseases are of interest to the layman from two economic standpoints:

I. At certain stages of their existence, certain insects have an economic value; for this reason their breeding is desirable and any plague which devastates their numbers should be combated. Pasteur was the pioneer in this line, not only being the discoverer of the first known bacillary insect disease, flacherie of the silk-worm, but he worked out an efficient method for its scientific control. His work is the more notable since he was handicapped by the lack of suitable methods of isolation and study of the organisms discovered.

II. Certain insects or their larvæ are at times veritable plagues laying waste valuable crops and causing serious hardships, even famine and epidemic disease resulting in many cases. Not infrequently these insects naturally become subject to microbial enemies which make heavy inroads on their numbers, thus checking the insect plague. Such an epizoötic occurred among the white grubs in Michigan in 1912.

The artificial employment of these microbial enemies naturally suggests itself as a means of voluntary control, and such experiments have been carried out successfully on a practical scale. One of the best examples of this is seen in the arrest of the locust epizoötic in Mexico and the Argentine Republic by the use of cultures of *B. acridiorum*.

Another thing worthy of note which has been mentioned many times by those working with microbial insect diseases, is the fact that these diseases seem to be almost explosive in character; an epizoötic among insects caused by a fungus disease is after a comparatively short time entirely wiped out and another disease takes its place; in many places bacterial diseases seem to have almost entirely supplanted the fungus diseases. This succession of diseases among insects takes place with such periodicity that those who are most intimately connected with their study, can predict very closely both the duration of the epizoötic in progress and the time intervening before the onset of the next one. This same periodicity takes place more or less among the more highly organized animals but the "explosive" character is greatly modified by the length of the life cycle.

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* Prepared by Zae Northrup Wyant, except paragraphs on "Miscellaneous Fungus Diseases" by C. Thom.

BACTERIAL DISEASE OF JUNE BEETLE LARVÆ, *Lachnosterna* spp.

Micrococcus nigrofaciens—Northrup*

HISTORY AND DISTRIBUTION.—The characteristics of this disease were noted in 1893 by Krassilstschik, Russia, but he did not consider it a disease. It is common everywhere in the United States that white grubs of this and related species are found; infected specimens have also been received from Porto Rico.

SYMPTOMS.—The normal larva is white, quite firm, covered with conspicuous hairs; the head is brown as are also the spiracles or breathing pores along either side. The diseased larva has black shiny spots, sharply circumscribed, located mainly along the joints of the legs, spiracles, and upon the dorsal or ventral segments of the white portion.

Badly diseased larvæ are almost entirely black or brownish black in color; the whole body often seems to be in a state of advanced putrefaction, yet the larva still shows life.

The progressive destructiveness of the disease is most marked in the affections of the legs. In some cases the infection begins at the tip of the leg and as it progresses, the leg, segment by segment, blackens and drops off, leaving the stumps shiny, black, and sometimes swollen in appearance; in other cases the infection occurs at one of the intermediate joints or at the joint nearest the body of the grub, the leg in time loosening and breaking off at the point of infection. Within certain limits neither the size nor the number of infected areas seems to affect the activity of the grub. Most grubs are very active unless badly infected with the disease.

CAUSAL ORGANISM.—Pure cultures of *M. nigrofaciens* show micrococci of varying sizes, 0.9μ and 1.2μ to 1.4μ diameter with dividing forms; occur singly, in pairs, threes (triangular), fours (tetrads or diamond shape), and clumps of more or less regular groupings, the individuals in a group arranged in a honeycomb-like order; chains of more than three never observed. Gram-positive; stains well with anilin dyes.

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* Northrup, Z. A bacterial disease of June beetle larvæ, *Lachnosterna* spp. Tech. Bul. 18. Mich. Exp. Sta., 1914.

Growth on agar abundant, beaded, flat, glistening, opaque, pale orange yellow, butyrous consistency, no odor. Cultures newly isolated are not pigmented and give only a moderate growth. Turbidity in broth, no ring or pellicle, no gas. Litmus milk is slightly reduced and acidified, no curd, yellowish deposit of bacterial cells. Gelatin is liquefied; dextrose, lactose and saccharose not fermented. Moderate indol production; nitrates reduced.

METHODS OF INFECTION.—Sterilized soil was inoculated with a broth culture of the micrococcus and in the soil were placed apparently uninfected larvæ, which were incised to imitate accidental abrasion. Characteristic lesions developed in two days at these points. Under natural conditions these larvæ bite one another, especially if they are very numerous in any one place; this may account for the rapid spread of the disease. *M. nigrofaciens* must be a common soil organism, especially where this disease is common. Parasitic insects or fungi may also aid in making infection possible.

Excessively wet soil favors the progress of the disease. Larvæ of *Allorhina nitida*, the southern June beetle, are susceptible to this infection but less so than the *Lachnosterna* spp. The American cockroach, *Periplaneta americana*, is also slightly susceptible, the infection limiting itself to the legs.

M. nigrofaciens does not lend itself readily to the control of the white grubs on account of the limiting environmental conditions.

FLACHERIE, AN INFECTIOUS DISEASE OF SILK-WORMS

Streptococcus bombycis—Cohn

HISTORY AND DISTRIBUTION.—Flacherie appeared in the silk industry as an epidemic at the end of the sixteenth century. It was again a serious epidemic about the year 1869 in the silk nurseries of southern France. Later it was found in Italy and other neighboring countries devoted to sericulture. In 1870 Pasteur recognized flacherie in silkworm as a disease of the silk-worm distinct from pébrine.

SYMPTOMS.—Diseased worms refuse to eat, become languid; after the fourth molt when they ordinarily climb up twigs and branches for the purpose of pupating, instead of spinning their cocoons they stretch out and remain motionless until death, or they may fall pendant, hanging by their pseudofeet. Worms when dead appear so very life-like that it is necessary to touch them in order to make sure that they are not living. From this appearance comes one of the names of this disease "morts-blancs."

After death they become soft in a short time and assume a blackish color in twenty-four to forty-eight hours. The body is then filled with a brownish fluid swarming with bacteria. Hundreds of worms in this condition show no polyhedral bodies (characteristic of pébrine). A glance is all that is necessary to distinguish worms dead of flacherie.

Causal Organism.—In the silk-worms as well as in culture *Streptococcus bombycis* forms short chains of small cocci, 0.89μ in diameter; the chains are from 5.01μ to 11.99μ long; stain well with anilin dyes and are Gram-positive.

In gelatin plate cultures, colonies are small, round, yellowish-gray, sharply contoured, finely granulated interior, gelatin not liquefied. Subsurface colonies have the same characteristics. Gelatin stab cultures are dull white, not liquefied. Agar colonies are small, round with a slightly undulating contour, deep brown in color, finely granulated, moist.

It is opalescent on glycerin agar and on ordinary agar when first isolated. In broth at 37° a marked turbidity is manifested after twelve hours without flocculence; after long standing the broth becomes clear. Potato cultures show an iridescence which later becomes a light gray. *Strept. bombycis* develops in milk without curdling it. It is a facultative anaerobe; the temperature optimum is 37° but it develops well at 20° also. The streptococcus retains its vitality for a long time in culture. It is destroyed at 65°–70° in fifteen minutes.

Methods of Infection.—Infection of the silk-worm takes place by means of food infected either with the excrement of sick individuals or with the dust of infected silk-worm nurseries of the year preceding.

When silk-worms show all the symptoms of flacherie, if they develop into moths the eggs laid by these moths are always infected. If any of the forms in which the silk-worm exists during its life cycle becomes infected it is sure to die before the cycle is completed.

Certain environmental conditions favor the rapid development of flacherie; high humidity due to an approaching storm or to keeping the worms enclosed in a practically air-tight cage prevents the transpiration which is so necessary to the worm after the fourth molt. Too many worms together often favors the progress of the disease.

Control.—Pasteur instituted the following means of producing healthy strains of the silk-worm; a small portion of the digestive cavity of a moth was abstracted with a scalpel, mixed with a little water and examined microscopically. If the moths did not contain the characteristic microörganism, the strain they came from might unhesitatingly be considered as suitable for seeding. The flacherie organism was as easily recognized as the pébrine corpuscle, but the infection was more difficult to prevent on account of the environmental conditions above mentioned.

Silkworms have been fed on mulberry leaves washed with water or an aqueous solution of lysoform, but a few sporadic cases of flacherie and emaciation occurred nevertheless.*

Phototaxy has been employed successfully in selecting larvæ of *Bombyx mori* most resistant to flacherie. Newly hatched larvæ immediately turn to the source of light, while this movement diminishes during the following days and disappears entirely at the end of the first stage. During the subsequent stages there is an inverse but less energetic movement and the larvæ tend to avoid the light. The larvæ which are most resistant to flacherie are those which from the time of their birth had travelled farthest.†

THE "JAPANESE GIPSY-MOTH DISEASE"

Streptococcus disparis n.sp.—Glaser‡

HISTORY AND DISTRIBUTION.—During the summer of 1915 a large series of eggs of the Japanese gipsy moth (*Porthetria dispar* L.) were hatched, which had been obtained for Glaser from Ogi, Japan. On reaching the third stage many of the caterpillars began to die of a peculiar disease which Glaser had never in previous years noticed in any of his American cultures. The infection later spread to the American race, and the most vigorous methods of isolation and disinfection had to be inaugurated in order to save most of the cultures from extinction. As the disease was very soon controlled, distinct in this respect from wilt (*polyhedral disease*), a bacterial origin was at once suspected. This disease was then studied in the belief that it might be used in combating the gipsy moth in the field. Many observations and tests show that this disease did not occur in this country prior to 1917.

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*Sacchi, Rosa. Partial disinfection of mulberry leaves in feeding silkworms. E.S.R. 39, pp. 560–561, 1918.

†Acqua, C. The use of phototaxy in selecting from the moment of their birth those larvæ of *Bombyx mori* most resistant to the disease flacherie. E.S.R. 38, p. 860, 1918.

‡Glaser, R. W. A new bacterial disease of gipsy-moth caterpillars. Jour. Agr. Res. 13, 1918, pp. 515–522.

SYMPTOMS.—When a caterpillar contracts this new disease it acquires a violent form of diarrhœa, loses its appetite, and finally ceases to eat. The insect seems to lose all muscular coördination and usually crawls to some elevated place, where it soon dies. After death it hangs in a flaccid manner by its prolegs, with an appearance of death from wilt. In contradistinction to wilt, however, the skin does not rupture, but is so tough that one can pick up and stretch the animal with considerable force before the skin breaks.

CAUSAL ORGANISM.—A microscopic examination of smears from the dead caterpillars readily precludes the possibility of wilt. Instead of polyhedra, large numbers of a streptococcus, *Streptococcus disparis* are present.

Strept. disparis has a diameter of less than 1μ; chains of 3 to 4μ frequent in liquid media; division in one plane; capsulated; non-motile; Gram-positive; stains readily.

Cultural characteristics are as follows: On nutrient agar slant, neutral, growth in five days at 35° scanty, beaded, flat, glistening, smooth, white, opaque, no odor, butyrous, medium unchanged. On potato agar slant, neutral, growth in five days at 35° abundant, spreading, flat, glistening, smooth, white, opaque, odor absent, butyrous, medium unchanged. Potato, growth moderate, spreading, flat, no odor, butyrous, color of medium unchanged. Gelatin stab, growth best at top, beaded, no liquefaction, medium unchanged. Nutrient broth, no ring or pellicle, slight clouding, clearing after fifteen days, slight sediment, no odor. Milk, coagulation delayed, extrusion of whey, color unchanged, no peptonization. Litmus milk, acid, prompt reduction, coagulation delayed, extrusion of whey, no peptonization. Dunham's peptone solution, clouding very slight, growth poor. Gelatin colonies, growth slow, colonies very small and majority under surface, surface colonies round, slightly convex, edge entire, no liquefaction. Nutrient agar colonies, growth slow, majority of colonies under surface and oblong, surface colonies round, smooth, convex, edge entire, internal structure finely granular, diameter 0.25 to 0.33 mm. Potato agar colonies, growth rapid, majority of colonies under surface and oblong, surface colonies round, smooth, convex, edge entire, internal structure finely granular, diameter 1 to 1.5 mm. No ammonia production. Nitrates not reduced. Indol and H_2S not produced. Acid but no gas is formed in the following carbohydrate broths used, extrose, levulose, saccharose, maltose, lactose, mannit, adonit, dulcit. Facultative anaerobe. Best media for cultivation 1.5 per cent neutral potato agar, and neutral nutrient bouillon containing 1 per cent of carbohydrate, especially saccharose, maltose or mannit. Pathogenic to caterpillars of the American, European and Japanese races of the gipsy moth (*Porthetria dispar L.*). Not pathogenic to silkworms (*Bombyx mori* L.) and army worms (*Cirphis unipuncta* Haworth) when fed *per os*. Guinea pigs, rabbits and human beings when fed pure cultures *per os* not affected. This organism is distinct culturally and biochemically from the organism, *Diplococcus lymantriæ* recently described by Paillot which is also parasitic in the gipsy moth caterpillar, and moreover is highly pathogenic to the caterpillars while *D. lymantriæ* is not very pathogenic.

Methods of Infection.—During the earlier stages of the disease when the caterpillars contract diarrhœa, the semiliquid fæces everywhere soil the food plants. This fecal matter is grossly contaminated with the streptococcus, and is the principal cause for the rapid spread of the infection.

Pathology.—Sections demonstrate that this bacterium, during the early stages of the disease, is found throughout the alimentary tract. Later, and especially after death, the intestinal epithelium disintegrates and ruptures, liberating the organisms into the body cavity where they invade practically all the tissues.

Microscopically striking changes can be noted in the muscle tissues even in the early stages of the disease. Normal muscle tissue shows clearly the striæ but in the early stages of the disease these show less clearly and the individual fibrillæ seem to be loosely arranged. Later stages of the disease show first an absence of the typical striated appearance due to the fact that the fibrillæ have lost their compactness and have separated from one another like threads of cotton; the arcolemma disintegrates gradually with the rest, and the nuclei of the cells lose their normal positions and become scattered. Up to this time it can be safely predicted that *Strept. disparis* will be found in the alimentary tract. Then finally, the muscle tissue disintegrates completely, the fibrillæ, etc., are no longer visible, and the whole simulates coagulated protein material with minute granules scattered throughout. When this stage in muscular disintegration has arrived, nearly all of the other tissues have likewise disintegrated more or less, and *Strept. disparis* may now be seen scattered everywhere.

Field experiments were conducted with *Strept. disparis* in sections of the gipsy-moth infested territory many times with success. In two places quite a severe epidemic was created. A large amount of work still is needed, however, to determine the relative importance of this method of combating the gipsy-moth.

BACTERIAL DISEASE OF LOCUSTS

Bacillus acridiorum—d'Herelle

HISTORY AND DISTRIBUTION.—Tropical and subtropical countries covering more than half the earth's surface suffer periodically from plagues of locusts of different species. Famine and its attendant, epidemic disease, follow in their wake and decimate the regions invaded. A bacterial epizoötic has become a natural means of control.

Bacillus acridiorum, the cause of the locust epizoötic, was discovered in Mexico in the state of Yucatan by F. d'Herelle. In 1909 a certain mortality was noted among the swarms which arrived from the south of the state where they winter; the following year the epizoötic was generalized and raged among a large number of bands; finally in 1911, all of the swarms which appeared were attacked, and in 1912, the locust invasion ceased. These particular locusts were the *Schistocerca pallens*.

SYMPTOMS.—The locusts which are attacked by the natural disease, present symptoms which are identical with those which are experimentally inoculated or contaminated *per os*. After a time of incubation, which varies from one to forty-eight hours according to the virulence of the bacillus, the age and individual resistance of the insect, and the environment (temperature especially), at first the contents of the chylific stomach become liquefied and assume a dark color resembling coagulated blood. The locust ceases to eat, becomes flabby, jumps awkwardly and hides itself under tufts of herbage. The intestinal contents next become liquefied; they are at first a clear yellow, later darkening little by little until they are blackish in color. At this stage a slight pressure upon the abdomen causes the liquefied intestinal contents to issue from the anus and the characteristic diarrhœa reveals itself on the vegetation which is fouled with the dejecta of the sick locusts. Some hours afterward the locust falls upon its side and the legs move spasmodically; the locust remains in this comatose state several minutes to several hours until death occurs. When the virulence of the coccobacillus is very high, very often the chylific stomach only presents the characteristic blackening; death occurs before the intestinal contents have undergone a modification. After death the insect putrefies very rapidly and the tegument becomes dark.

The intestinal content of locusts attacked with this disease shows microscopically practically a pure culture of this bacillus. The intestinal contents of healthy locusts are poor in bacteria, sometimes seemingly aseptic. Among the saprophytes found, the most common is a motile, Gram-positive coccobacillus which causes death of locusts by injection but never by ingestion. It is distinguishable from the specific coccobacillus by the disagreeable odor which it produces in the locusts or in culture media. Sometimes staphylococci are found, rarely *B. subtilis;* only one saprophyte per hundred of the specific bacillus renders the isolation of the latter very simple.

All of the tissues of the locust are invaded by this bacillus as has been proved microscopically. A pure culture can be obtained from the blood at the same time that the intestinal contents are attacked, thus *B. acridiorum* produces a veritable septicemia.

Causal Organism.—*B. acridiorum*, the causal organism of the Mexican locust epizoötic is a short, slightly ovoid bacillus, decidedly polymorphous; in the same culture coccus forms of about 0.6μ are found beside of forms plainly bacilli, 0.4μ to 0.6μ by 0.9μ to 1.5μ; actively motile possessing peritrichic flagella; Gram-negative but stains readily with anilin dyes; the bacillus takes the stain most deeply at the poles, especially if Ziehl's carbol-fuchsin is used for one to two seconds.

Facultative anaerobe; cultures grow readily from 16° to 43° in all ordinary media, even in Raulin's medium. It develops very rapidly at 37° in broth, turbidity appearing at about the fourth hour. A delicate membrane is formed on broth which clears only after three weeks, leaving a heavy sediment. Young agar colonies are circular and have a waxy appearance; they grow rapidly, being plainly visible after twelve hours; after eighteen hours, they are 2–3 mm. in diameter. Subsurface colonies are small, spherical, whitish and opaque. Gelatin is not liquefied. Milk is coagulated and rendered strongly alkaline. Grows abundantly on potato having a creamy appearance; the culture in the water at the bottom of the tube is so dense that the liquid becomes sirupy and has a strong alkaline reaction. Dextrose, levulose, maltose and galactose are fermented; the inoculated medium containing one of these sugars becomes acid at first, then alkaline. This alkalinity is due to the formation of ammonia. *B. acridiorum* has lived over two years in sealed tubes.

Methods of Infection.—Natural.—There are several natural methods by which the epizoötic is spread. Sick locusts or nymphs leave their infectious liquid dejecta on the vegetation, the other locusts eat the contaminated herbage, contract the disease and in turn infect new plants thus continuing the cycle. With certain species of locusts, the *Schistocerca* for example, another very important mode of contagion exists: when one of their number becomes weak or where vegetation is scarce both the nymphs and the adults eat one another.

At the time of depositing the eggs, if the female or even the male is diseased, the eggs will be forcibly soiled with the liquid of the diarrhœa and the bacillus will be conserved up to the time of hatching upon the eggs or in the mucilaginous matter surrounding the eggs which the locust has provided for their protection.

A certain number of locusts among every swarm act as healthy "carriers." Carriers among nymphs have never been found.

The period of life of the insect affects its resistance. The adult locust is individually much more susceptible than the nymph. The habits of life of each, however, have a great influence. The nymphs are continually in contact with the vegetation and with each other as they march in very dense columns; they are endowed with a voracious appetite and undergo in the short period of their larval life, five molts, which are the periods of their least resistance. The winged locusts, to the contrary, passing a large part of their life in the air, are only rarely pressed one against the other, except, for example, when the weather is cold; they also eat much less than the nymph, thus the epizoötic will have a greater tendency to become generalized among the bands of nymphs than among the swarms of adult locusts.

The age of the nymph or locust influences its resistance; the young nymphs have a maximum resistance, but this decreases gradually, reaching its minimum at the time of the last molt; the adult locust has its minimum of resistance at the egg-laying period.

The period of the molt is not a means of protection against this type of disease, which is a generalized septicemia.

Artificial.—The virulence of *B. acridiorum* decreases very rapidly in culture and in order to obtain the desired destruction of locusts it is absolutely necessary to employ cultures of the highest possible virulence as an attenuated virus immunizes the locust and renders it refractory to a culture of the highest virulence when applied later.

The virulence is increased by successive passages through locusts or nymphs; twelve series of passages are made using twelve locusts in each series. The culture to be rejuvenated is mixed with a few cubic centimeters of sterile water or broth. Injections are made with a syringe having a very fine sharp-pointed needle. The insect is seized with the left hand, the ventral portion toward the operator, and the needle of the syringe inserted between the second and third anterior abdominal segments at the point of intersection with one of the longitudinal ridges, horizontally in the direction of the head to a depth of about 3 mm. for an adult insect, a little less for a nymph. The point of the needle should enter the abdominal cavity, not merely pierce the tegument as in the latter case the effect would be nil. If the needle is inserted too deep the internal organs will be injured. A very fine-pointed bent pipette could be employed equally well. One or two drops of the emulsion of the old culture are injected.

As soon as the locusts in the first series become sick or preferably are nearly dead, press the abdomen between the fingers and collect in a watch glass the blackish liquid which issues from the anus. Inject a drop of this liquid into the abdominal cavity of the second series of locusts, following the same technic and observing the same precautions as for those of the first series. These insects will die in a shorter period of time. Obtain as previously, in a watch glass the intestinal liquid of three or four of the first dead locusts of the second series, dilute half with water and sterile broth and inoculate the third series. To inoculate the fourth series, use the intestinal liquid of the first dead of the third series diluted to a third; a fifth series with the liquid diluted to a fourth and continue with the series in this way. It is exceptional that it will be necessary to proceed further than the twelve series. The virulence of *B. acridiorum* is increased sufficiently if death occurs eight hours after injection. One-hundredth of a cubic centimeter of virus at its maximum virulence injected into a locust will cause the characteristic diarrhœa in two hours and death an hour later. This method of increasing the virulence takes five to six days and this period of time has to be taken into consideration when it is necessary to employ the culture on a practical scale.

When the acridian to be infected belongs to a different species than that for which the virulence of *B. acridiorum* has been previously augmented, a large number of passages may be necessary as a culture virulent for one species may not be able to infect another species. In one case fifty-two passages were necessary in order to kill *Stauronautus maroccanas* (Algeria) in eight hours while for the same insect at Cyprus only twelve passages were necessary. It is also desirable that the first few series consist of a large number of insects, as there will be apt to be some which will be more sensitive to the virus. Their natural resistance can be weakened by fasting for several days before inoculation. The intestinal contents should not be diluted until the virus will kill within fifteen hours.

When the virulence is sufficiently increased, the specific bacillus is isolated by means of an agar slant or plate and cultivated for twenty-four to thirty-six hours at room temperature; it may then be isolated if the virus is to be conserved; if desired for direct infection experiments, it may be placed directly in broth. The broths used is made as follows:

Water	1,000 c.c.	Boil, alkalinize slightly and filter; place in bottles, plug with cotton, cover mouth and neck with parchment paper cap, and sterilize at 120° for thirty minutes.
Peptone	40 gr.	
Salt	5 gr.	
Gelatin	30 gr.	
Dextrose	5 gr.	

The gelatin serves to fix the organisms in place when the culture is sprayed on the vegetation, and on account of the dextrose the plants are greedily devoured by the locusts.

It is necessary to remember that the virulence of *B. acridiorum* lowers very rapidly in culture and is attenuated likewise by re-transplantations, so that a broth culture so prepared should be used within two or three days at the utmost. If the campaign against the locusts lasts for several months or the regions invaded are extensive, it will be necessary to continue the series of passages during the campaign in order to have on hand a virus of maximum efficiency. It is best to make two or three more passages than necessary, rather than too few, for in the latter case the results of a whole campaign may be nullified.

The material necessary for a campaign against the locusts consists of a *new* spray pump, preferably tinned inside, such as is used in spraying fruit trees, and the bottles containing the pure broth culture of *B. acridiorum* at a maximum virulence. A used spray pump should never be employed as it is practically impossible to free it from the antiseptic contained. The pure culture should be used as soon as it shows turbidity. Broth cultures should never be used which have a putrefactive odor.

In practice it is generally necessary to infect the greatest number of locusts in the largest possible number of bands in order to exterminate them with certainty in as short a time as possible. The quantity of broth culture to be sprayed varies with the area covered by the nymphs or locusts. One liter per hectare is sufficient in all cases. For large areas, *e.g.*, 100–200 hectares, spray over twenty different places, using one-half liter each time, taking in all ten liters. It is better to spray over a large area rather than all in one place, choosing places where the nymphs or locusts are in largest numbers, and always spraying the type of plants preferred and in advance of where they are eating. Spraying should be done in the early morning or preferably in the evening towards sunset. The heat and especially the bright light of day rapidly attenuate the virulence of the bacillus. If necessary to spray in the middle of the day, shady spots should be chosen.

The virulence and vitality of *B. acridiorum* has been conserved in the dejecta and dried cadavers for seven months while in culture the virulence, especially, is lost rapidly.

A very hard rain will inhibit the progress of an epizoötic for several days. The rain washes the dejecta of the locusts from the contaminated vegetation, hindering this mode of contagion; the epizoötic little by little regains its normal activity. A rain of short duration, to the contrary, seems to favor the progress of the disease.

A curious phenomenon takes place when a band of infected nymphs meet a river in the course of their route. On the near bank is found an actual heap of cadavers, on the opposite bank likewise but they are very much less in number. The epizoötic seems to be completely checked; it recommences only after several days when it takes its normal course. This is explained by the fact that all the nymphs already badly diseased are not strong enough to make the necessary effort to cross the stream and die without surmounting the obstacle; those which were only slightly diseased could pass it but were so enfeebled by their effort that they died on the opposite bank. Thus the colony which pursues its march is composed only of healthy insects and of several nymphs in which the infection has hardly begun.

The duration of an epizoötic is impossible to predict for all species of insects and under all conditions; as a general rule it will last several days, most often several weeks, rarely several months. The duration of an epizoötic, however, is of little importance; the object is to cause such a reduction in the number of the locusts that these insects will cease to be a plague.

To spread the epizoötic to great distances, care should be taken to infect the winged adults. Some species of locusts are more sedentary than others, it follows that the more sedentary a species is, the more necessary to multiply the foci of infection.

In order to ascertain whether the epizoötic is progressing, gather one hundred locusts from different parts of the swarm and by pressing their abdomens, see how many show the characteristic diarrhœa. Those insects showing diarrhœa one day will be dead the next.

Certain peculiarities were observed during the course of an epizoötic. In swarms infected a little while before egg-laying, numerous females lay eggs which never reach maturity; others never reach the laying stage and the eggs are transformed within the body to a blackish mass. Such bands were annihilated several days afterward. In bands of nymphs infected several days before the last molt are found numerous abnormal adults with poorly developed wings only half their ordinary length which prevent them from flying, and further a microscopic examination of the genital organs shows complete atrophy.

SUSCEPTIBLE INSECTS.—I. Acridians.—*B. acridiorum* should be pathogenic for all acridians. The following species are susceptible: *Schistocerca americana* (or *pallens*).—Natural epizoötic in Yucatan in 1908–1911, induced in the Argentine Republic in 1912.

Caloptenus sp?.—Epizoötic induced in December 1912 in the region of Rio Negro, Argentine Republic.

Stauronautus maroccanus.—Epizoötic induced in 1913 in Algeria in the province of Oran, and in the isle of Cyprus.

Schistocerca paranensis is killed by *B. acridiorum*. (Argentine Republic.)

Gryllus pennsylvanicus, one of the common field crickets is susceptible. (DuPorte and Vanderleck).

Zonocercus elegans, the so-called "elegant grasshopper" of South Africa, a non-migratory species, was used in inoculation experiments with this bacillus. It was concluded that this disease at best could be employed only as a supplementary measure in dealing with the invasion of these insects under conditions that prevail in South Africa.

The Philippine locust, *Pachytylus migratoroides*, has given negative results with *B. acridiorum*. (Mackie).

II. ANTS.—A species of small ant near Paris was annihilated in 1911 by *B. acridiorum*.

Selenopsis gemminata, near Buenos Aires was annihilated in 1912. Several drops of the culture were placed in each ant hill.

Atta sexdens, a veritable plague in the tropical and sub-tropical countries was annihilated at Chaco and Tucuman after the virulence had been increased for this species of ant by many passages.

III. CATERPILLARS.—A field of cotton which was being ravaged by caterpillars was treated with *B. acridiorum*. Four days afterward all the caterpillars were dead while a neighboring field; treated simultaneously with Paris green, still contained many living caterpillars.

The yellow bear caterpillar (*Spilosoma*) *Diacrisia virginica*) has been found to be susceptible. (DuPorte and Vanderleck).

B. acridiorum does not attack the silk-worm, *Bombyx mori;* it kills the cockchafer, *Melolontha vulgaris*, by injection but not by ingestion.

Birds and mammals in general are immune to this bacillus. One notable exception is the sewer rat which dies from generalized septicemia a few hours after injection. The rat was immune to cultures ingested.

IV. BEETLES.—The Colorado potato beetle, both larvæ and adults, is not susceptible. (DuPorte and Vanderleck).

BACILLARY SEPTICEMIA OF THE CATERPILLARS OF *Arctia caja* L.

Bacillus cajæ—Picard and Blanc*

HISTORY AND DISTRIBUTION.—In 1913 the vineyards of central France were almost completely destroyed by two diseases; one of these was a fungus disease caused by *Empusa aulicæ*, the other was a septicemia of bacillary origin.

SYMPTOMS.—The caterpillars become flaccid and emit a nauseating odor; their digestive tube contains only a clear liquid free from all organisms. The blood contains a pure culture of a bacillus with which the disease has been produced artificially.

CAUSAL ORGANISM.—*B. cajæ* is a slightly oval bacillus, about 1.5μ in length; motile; Gram negative; stains deeply with crystal violet; treated by Pappenheim's method it shows a characteristic bi-polar stain.

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*Picard, F. and Blanc, G. R. On a bacillary septicemia of caterpillars of *Arctia caja* L. Compt. rend. acad. sci. 156, 1913, pp. 1334–1336.

Broth cultures develop in twelve hours at 15°–35° with an optimum of 25°; from these the odor of H_2S is perceptible; in twenty-four hours broth cultures have a green fluorescence which is more marked at 25° than at 15° or at 35°. Grows rapidly on both gelatin and agar showing a green fluorescence, the former is liquefied. Growth on potato is meager, showing only after forty-eight hours and producing no pigment.

METHODS OF INFECTION.—Artificial.—Caterpillars of *Arctia caja* inoculated in one of their feet by means of a fine needle dipped in virulent blood or in a broth culture, die regularly in three days at 15°, manifesting in their blood swarms of the specific bacteria. If kept at 25° they die in twelve to twenty-four hours. The blood of the caterpillars kept at the latter temperature appears to be the more virulent.

Caterpillars receiving several drops of culture by means of a pipette introduced into the pharynx, die in twelve hours at 25° with their blood invaded by the bacteria. This suggests a possible practical application.

SUSCEPTIBLE INSECTS AND OTHER ANIMALS.—Caterpillars of *Porthesia chrysorrhea* are very sensitive to *B. cajæ* and die on inoculation in twenty-four to forty-eight hours.

The following Coleoptera: *Hydrophilus pistaceus*, *Dyticus pisanus*, *Cybister laterimarginalis*, *Colymbetes fuscus* are not killed by inoculation; nor are the following Hemiptera: *Notonecta glauca, Nipa cinerea, Ranatra linearis.*

The white rat is not sensitive to intraperitoneal injection of 1 c.c. of a twenty-four-hour broth culture. The tree frog, *Hila arborea*, dies by inoculation, with the same culture, into the lymphatic sacs in twenty-four to forty-eight hours with the blood invaded by numerous organisms. The blood of dying caterpillars is more virulent for the tree frog than broth cultures; 0.5 c.c. injected into the lymphatic sacs causes the death of the batrachian in twelve hours with an intense bacillary septicemia.

B. cajæ seems to belong to the same group as d'Herelle's *B. acridiorum.* It is distinguished from it however by several characteristics, both biological and pathological, being a parasite of the blood of the caterpillars whereas, according to d'Herelle, the site of affection in the diseased locusts is in the digestive tract.

GRAPHITOSIS*

Bacillus tracheitis or graphitosis—Krassilstschik

HISTORY AND DISTRIBUTION.—This disease together with a bacterial septicemia was noted among the *Lamellicornia* in 1893 in the southeast of Russia by Krassilstschik. He states that the larvæ from the *Lamellicornia* which formerly died *en masse* of muscardine,† die of this disease very seldom in recent years. Bacterial parasites seem to have replaced it in this part of Russia, and this is distinctly advantageous since the bacterial diseases are much more destructive than any of the species of muscardine.

SYMPTOMS.—At first the larva is entirely pure white, then several legs change to a bright yellow color, next to a yellow brown. Little by little this coloration extends over all the legs. Later on both sides of the larva, characteristically in the region of the spiracles and around them, the skin takes on a grayish hue which gradually deepens. The larva is generally living at this stage but appears to be diseased. This grayish coloration extends toward the back, and the anterior part of the larva then gradually becomes gray also. At this stage the larva is generally dead. After death, the gray color, spreading characteristically from the spiracles, deepens considerably, extending all over the skin, finally acquiring a tint resembling that of polished graphite, whence its name "graphitosis." This coloration is very characteristic for the larva which die of this disease; it is only very rarely that the cadaver is of a brownish shade.

When the infection first shows in the legs, the movements are not inhibited in any way, but when the graying around the spiracles sets in, the larva becomes comatose yet still responding to exterior excitations. It soon dies, retaining its characteristic curve but gradually becoming limp and soft and decreasing in size, length, etc.

CAUSAL ORGANISM.—The bacillus of graphitosis is from 2μ to 2.2μ long having a diameter of more than half its length; spores are produced; very motile, movements quick and rapid; occurs generally in pairs from 3.6μ to 4.6μ long; long filaments not produced; the longest do not exceed 7μ to 9μ which corresponds to two pairs of bacilli end to end. Aerobic; shrinks perceptibly when treated with Gram's stain, almost to half its size. Stained bacilli show unstained spots (spores).

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* *Bacillary Diseases of Lamellicornia.*—In 1893 Krassilstschik described two bacterial diseases attacking the larvæ of the following insects: *Rhizotrogus solstitialis, Melolontha vulgaris, Anisoplia austriaca, crucifera, and fruticola, Cetonia* sp. and a larva belonging to the *Geotrupini* (*Lethrus?* sp.).

† Krassilstschik, I. Graphitosis and Septicemia of Insects. Memoires Soc. Zool. en France, t. vi, pp. 245–285, 1893.

In plate cultures *B. tracheitis* develops into small circular colonies 0.25 to 0.75 mm. in diameter, which are covered with tubercles when the colony grows in gelatin. Gelatin colonies are finely granular of a deep brownish-yellow color, opaque center surrounded by a transparent ring. Gelatin is liquefied in twenty-four to forty-eight hours. Gelatin stab cultures are typical, having a cup-shaped hollow funnel at the surface, a short empty stem; then the culture grows in the depth of the gelatin, liquefying it in the shape of a carrot, later becoming the shape of an inverted bottle; the culture is seen along the original path of the stab as a zigzag line which later forms a compact cream-colored deposit as the gelatin becomes entirely liquefied; these cultures have the odor of the white of an egg. Broth cultures are clear the first twenty-four hours; after that they become turbid and a pellicle forms which thickens with age, sediment compact; cultures become wholly transparent in four to six months.

METHODS OF INFECTION.—Washing the larvæ of the *Lamellicorniæ* with the natural virus of graphitosis kills 16.6 per cent to 100 per cent but an augmented virus gives 100 per cent mortality. The injection under the skin of a very small drop of graphitosis blood is always fatal for the larva even if the virus is weak.

B. tracheitis multiplies first in the blood system, then fills the Malpighian tubes, next characteristically in the trachea and then in the fatty bodies; the trachea becomes typically filled with black amorphous granules from whence the name of this organism; the fat cells are attacked. The intima of the muscles, trachea and other organs is covered with bacilli, which however do not penetrate the organs themselves.

AMERICAN FOUL BROOD

Bacillus larvæ—White*

HISTORY AND DISTRIBUTION.—American foul brood is the prevalent disease among bees in America and is distributed through all parts of the United States, in Ontario, Canada, Switzerland, New Zealand, Germany, England, and France and it is probable that it has a much wider geographical distribution.

SYMPTOMS.—American foul brood or simply "foul brood" usually shows itself in the larva just about the time that the larva fills the cell and after it has ceased feeding and has begun pupation.

At this time it is sealed over in the comb. The first indication of

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* White, G. F. American foul brood. Bul. 809. Bur. of Ent. U. S. Dept. of Agr., 1920.

the infection is a slight brownish discoloration and the loss of the well-rounded appearance of the normal larva. At this stage the disease is not usually recognized by the beekeeper. The larva gradually sinks down in the cell and becomes darker in color, and the posterior end lies against the bottom of the cell. Frequently the segmentation of the larva is clearly marked. By the time it has partially dried down and become quite dark brown (coffee colored) the most typical characteristic of this disease manifests itself. If a match stick or tooth-pick is inserted into the decaying mass and withdrawn the larval remains adhere to it and are drawn out into a thread, which sometimes extends for several inches before breaking.

This ropiness is the chief characteristic used by the beekeeper in diagnosing this disease. The larva continues to dry down and gradually loses its ropiness until it finally becomes a mere scale on the lower side wall and base of the cell.

The scale formed by the dried-down larvæ adheres tightly to the cell and can be removed with difficulty from the cell wall. The scales can best be observed when the comb is held with the top inclined toward the observer so that a bright light strikes the lower side wall. A very characteristic and usually penetrating odor is often noticeable in the decaying larvæ. This can perhaps best be likened to the odor of heated glue.

The majority of the larvæ which die of this disease are attacked after being sealed in the cells. The cappings are often entirely removed by the bees, but when they are left they usually become sunken and frequently perforated. As the healthy brood emerges the comb shows the scattered sunken cappings covering dead larvæ, giving it a characteristic appearance.

Pupæ also may die of this disease, in which case they too, dry down, become ropy, and have the characteristic odor and color. The tongue frequently adheres to the upper side wall and often remains there even after the pupa has dried down to a scale. Younger unsealed larvæ are sometimes affected. Usually the disease attacks only worker broods, but occasional cases are found in which queen and drone broods are diseased. It is not certain that race of bees, season, or climate have any effect on the virulence of this disease, except that in warmer climates where the breeding season is prolonged, the rapidity of devastation is more marked.

Causal Organism.—*Bacillus larvæ* is a slender rod with ends slightly rounded and with a tendency to grow in chains. The length varies greatly, depending for the most part upon the medium used for its cultivation. It varies from 2.5 to 5μ in length, and is about 0.5μ in breadth when grown on the surface of brood-filtrate agar. In a liquid medium it is usually much longer, frequently becoming filamentous. Giant whips occur in large numbers, especially in the condensation water of brood-filtrate agar slant cultures. They are also present in decaying larvæ dead of American foul brood. The flagella are peritrichic: when twisted into giant whips, these corkscrew-like structures vary widely in their dimensions from scarcely visible coiled filaments to bodies several microns in diameter. Motility moderate in young cultures from the surface of brood-filtrate agar, sluggish in liquid cultures. Spores formed about the third day on brood-filtrate agar; median, causing a spindle-shaped enlargement of the rod; free spores measure about 0.6 by 1.3μ. Few or no spores are formed in liquid media, deep in solid media, and on media containing glycerin, mannit, or dextrose. Some of the other sugars, and also honey inhibit spore formation. The rods stain readily with ordinary anilin dyes, and are Gram positive.

B. larvæ is cultivated with difficulty, growing best on media made as the ordinary laboratory media, substituting bee larvæ for meat, or on egg-yolk-suspension agar. Bee larvæ agar, however, is limited in its usefulness on account of the large amount of brood required in its preparation. The unheated egg-yolk agar is prepared as follows: immerse *fresh* eggs in a disinfecting solution, break the shell, pour off the white, and drop the yolk into a flask containing about 70 cc. of sterile water; agitate the flask to make a homogeneous suspension of the yolk, and with a sterile pipette transfer the aqueous suspension to sterile tubes and store until needed. For use: melt tubes of agar and cool to about 50°, add about 1 cc. of egg-yolk-suspension to each 5 c.c. of melted agar, and either incline and allow to harden, or use immediately for plating as desired. A more detailed description of the technic employed in making both of the special media necessary for the cultivation of *B. larvæ* will be found in the bulletin by White. *Bacillus larvæ* is present in practically pure cultures in brood dead of American foul brood, so this organism can be readily obtained from brood dead of this disease by heating the spore-containing material in aqueous suspension at 100° for one or two minutes, and plating in bee-larvæ agar or egg-yolk suspension agar. When bee-larvæ agar alone is employed and inoculations are made with spores, following Liborious' method for anaerobes, growth as a rule appears more often near to than on the surface, indicating partial anaerobiosis. Sub-cultures on brood-filtrate agar and egg-yolk-suspension agar or their combination yield abundant surface growth.

Brood-filtrate agar slant, growth rapid, being moderate to heavy in twenty-four hours, somewhat spreading, grayish white and slightly viscid; has a more or less uniform border, a smooth surface, and a ground glass appearance. Older cultures are less prominent than the younger ones. Brood-filtrate agar plates, surface colonies vary in size depending upon the number present. When well isolated they not infrequently spread, attaining a diameter of 1 cm. or more; growth only slightly raised, smooth surface, ground glass appearance, with a clearly defined, uniform border. Deep colonies vary from lenticular to irregular in form with filamentous outgrowths from portions of their surface. No visible gas, but slight acidity in carbohydrate broths to which a little brood filtrate or egg suspension has been added. Gelatin no growth in plain or in brood-filtrate gelatin at temperatures at which it remains solid.

The more resistant spores of *B. larvæ* require 100° for eleven minutes to destroy them, and when suspended in honey require a half hour or more. Five per cent. carbolic acid is resisted for months, 1–1000 mercuric chloride for days, 10 per cent. formalin for hours, and 20 per cent. formalin for thirty minutes, in each case at room temperature. In fact, most destructive agencies are resisted by these spores. Drying at room temperature has been resisted for nine years, and it is most likely that they will remain viable and virulent for a very much longer period. Dry spores exposed to direct sunlight are killed in from twenty-eight to forty-one hours. Four to six weeks are required for destruction when suspended in honey and exposed to the direct rays of the sun, but if shielded from direct sunlight, the spores remain alive and virulent for more than a year. The destructive effects of fermentation have been resisted for more than seven weeks at incubator and outdoor temperatures, and it is likely that a much longer period could have been withstood.

B. larvæ is pathogenic for the larval, prepupal, or early pupal stages of the brood of honey bees. Adult bees, rabbits, guinea pigs, rats and humans are not susceptible to infection.

METHODS OF INFECTION.—Natural.—American foul brood infection is transmitted primarily through the food of bees; possibly at times to some extent through their water supply. Robbing from the diseased colonies of the apiary, or from neighboring apiaries, is the most likely mode by which the disease is transmitted in nature. The placing of brood combs containing diseased brood with healthy colonies will also result in the transmission of the disease. It is not likely that infection ever occurs through the medium of flowers. Queens and drones have been presumably overestimated at times as possible sources of infection. It has not been determined as yet whether American foul brood is ever transmitted by them. The clothing or hands of those about an apiary or handling the bees are not fruitful sources for the transmission of the disease. The hive tool, if brought in direct contact with dead larvæ in testing for the presence of disease, might serve to transmit infection, but during the usual manipulation it would not. Other tools and bee supplies generally about an infected apiary will not transmit the infection in the absence of robbing from those sources.

Artificial.—American foul brood can be communicated by feeding to a healthy colony the scales from combs which had contained brood affected with American foul brood; likewise when these scales are placed in ordinary meat broth, incubated twenty-four hours and then heated to 65° for twenty minutes; infection in this case is due to the presence of spores. Pure cultures of *B. larvæ* mixed with sterile sugar sirup and fed to healthy colonies produce the disease within three weeks. *B. larvæ* can be obtained in pure culture from such diseased larvæ.

A fact of special importance not only in the technic of making studies but also in the control of the disease is that colonies in which the disease has been produced through artificial inoculation can be kept in the experimental apiary without transmitting the disease to others.

B. larvæ may be obtained in large quantities suitable for experimental inoculation by diluting and filtering the crushed bodies of bee larvæ through a Berkefeld or other fine filter.

CONTROL.—The treatment of an infectious bee disease consists primarily in the elimination or the removal of the cause of the disease. Effort is not made to save the larvæ already dead or dying, but to stop further devastation by removing all material capable of transmitting the cause of the trouble. The swarm is transferred from the infected hive to a clean disinfected hive; the infected combs from the old hive should either be burned, melted, or boiled thoroughly before the wax is fit for use again. The honey taken from the infected hive should be buried or at least removed so that no bees can use it for food. This treatment may have to be repeated before the disease is under control. Brood from badly diseased colonies should be burned, buried or otherwise destroyed at once. Combs even if they appear white and clean should be melted. Chemical disinfectants should not be relied upon. Infected hives should be burned over inside with a gasoline or oil torch.

SEPTICEMIA OF THE COCKCHAFER, *Melolontha vulgaris*

Bacillus melolonthæ—Chatton*

HISTORY.—In May, 1912, while studying the effect of d'Herelle's *B. acridiorum* on the cockchafer, Chatton noticed that the cockchafers were dying from a spontaneous septicemia; this he found later was due to a coccobacillus which he named *B. melolonthæ*.

SYMPTOMS.—No symptoms are noted.

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* Chatton, E.: Spontaneous septicemia in the cockchafer and the silk worm due to coccobacilli. Compt. rend. acad. sci. 156, 1913, pp. 1707-1709.

CAUSAL ORGANISM.—*B. melolonthæ* resembles *B. acridiorum* of d'Herelle with the exception of the following characteristics: the bacillus is longer, and in agar culture produces a green fluorescence in five to six days. It is distinguished from the bacillus of d'Herelle in addition by its pathogenic action on the silk-worm, *Bombyx mori.*

METHODS OF INFECTION.—Injected into the general cavity, *B. melolonthæ* kills the cockchafer in twelve to thirty-six hours, and where its virulence has been augmented by several passages through this insect, always in less than twenty-four hours, but *per os*, it is as inactive as *B. acridiorum.* Seventy-five per cent of healthy cockchafers show the presence of *B. melolonthæ* in their digestive tube, sometimes in massive culture. This is always the case with cockchafers affected with septicemia.

This blood disease seems to be of intestinal origin however, as with the locust. *B. melolonthæ*, a common parasite of the intestine of the cockchafer passes into the general cavity only under special conditions yet unknown. When this organism is removed from the intestine and injected into the general cavity, septicemia is produced.

It is as virulent for the silk-worm by injection as for the cockchafer, and as inactive by ingestion.

EUROPEAN FOUL BROOD

Bacillus pluton—White*

HISTORY AND DISTRIBUTION.—This type of foul brood, sometimes known as "black brood," or "New York bee disease" is not nearly as wide spread in the United States as is American foul brood, but in certain parts of the country it has caused enormous losses. It is spread over England, Germany, Switzerland and other parts of Europe and has been noted many times during the last decade.

SYMPTOMS.—The presence of disease can usually be detected in an experimental colony during the week that feeding is begun. The first indication of it may be that only a portion of a larva is seen in a cell, the remaining portion having been removed by the bees. Aside from an observation of this kind, the earliest indication one gets from the macroscopic examination is that sick larvæ are found among the uncapped brood.

Sick larvæ manifest certain symptoms during the course of the disease by which its presence can be diagnosed while the larvæ are still alive. The length of time that a developing bee is sick of European foul brood is variable. In general, the three days just preceding the time when a larva would ordinarily be capped, is the most favorable period for making a diagnosis from the gross examination alone. Healthy larvæ at a certain age when slightly magnified show a peristalsis-like motion of their bodies, but larvæ of this same age when sick frequently exhibit a marked peristalsis which can easily be seen with the unaided eye. Diseased larvæ may show a yellowish tint or appear transparent instead of the glistening white or bluish white of healthy larvæ.

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* White G. F.: European foul brood. Bul. 810, B of Ent. U. S. Dept. of Agr. 1920.

Another symptom often serves for diagnosis. In a healthy larva a pollen-colored mass is frequently plainly seen through the transparent area along the dorsal median line. If this intestinal mass appears white or yellowish white, the presence of European foul brood is almost certain. This may be often more plainly observed if the larva is removed from the cell with forceps.

European foul brood may be positively diagnosed in living larvæ of a favorable age and condition by the following method: Remove the larva to be tested from the cell and place it upon glass, preferably with a dark background; with a dissecting needle in each hand and with their points near together, pierce with both needles so as to tear the body wall crosswise, and continue to separate the two portions of the larva. If the larva is diseased, and one is successful, it will be found that the intestinal content will be stripped from and pulled out of the posterior and blind end of the canal. The intestinal content of healthy living larvæ cannot be removed in this way. The force which is applied in pulling the mass from the intestine frequently causes the typical transparent, mucus-like substance surrounding the central mass to stretch and the enclosed whitish substance to break into segments; this appearance is very characteristic.

If the disease is more advanced, a portion of the intestinal content may flow out in the form of a sac, the wall of which is very easily broken. When broken the content of this sac-like structure will flow out as a rather thin whitish or yellowish white fluid containing small whitish granules that vary in size. If the disease is far advanced and the larva probably dead, the enveloping substance of the intestinal content is so easily broken that often only the whitish or yellowish-white fluid flows from the ruptured wall of the larva.

Dying larvæ diseased with European foul brood frequently show the segments of the body marked off less distinctly than living healthy larvæ.

Causal Organism.—*B. pluton*, the organism of European foul brood, is a small, non-spore-forming organism, sharply pointed at one or both ends, about 1μ long and less than 0.5μ in breadth, on the average; occurs frequently in pairs; single individuals vary very markedly in size and shape.

This organism has never been cultivated, but sections of larvæ in various stages of the disease show *B. pluton* to be the first invader of healthy larvæ. *B. pluton* gains entrance to the larva by way of the mouth. The growth and multiplication of the parasite take place within the stomach and do not, during the life of the larva get beyond the peritrophic membrane. The tissues therefore, are not invaded by it. The secondary invaders in European foul brood, *B. alvei*, *Strept. apis*, *Bact. eurydice*, and *B. orpheus*, rarely, if ever, invade the tissues until the larva is dead or nearly so. In American foul brood, practically speaking, there are no secondary invaders, either during the life of the infected larva, or during the decay of the remains.

Experimentally, *B. pluton* suspended in water, was killed at approximately 63° in ten minutes, but when suspended in honey, 79° for ten minutes had to be applied. Dried, *B. pluton* remained alive and virulent for approximately a year. In the dry state direct sunlight was not destructive until after twenty-one to thirty-one hours, but when suspended in water, only five to six hours were required for destruction, and when suspended in honey exposure for from three to four hours was fatal.

In the presence of fermentative processes in a 10 per cent sugar solution *B. pluton* was destroyed in from three to five days at incubator temperature and in from eleven to twenty-one days at room temperature. In a fermenting honey solution outdoors, it was still alive and virulent after one month; in undiluted honey at room temperature *B. pluton* ceased to be virulent in from three to seven months. Mixed with pollen, this bacillus remained alive and virulent for more than seven months at room temperature and for more than ten months at refrigerator temperature. Putrefactive processes were destructive to *B. pluton* in from seven to thirteen days at incubator temperature, in from twenty-one to thirty-five days at room temperature, while at outdoor temperature it remained alive and virulent for more than forty days, the maximum time not being determined.

B. pluton was destroyed by 0.5 per cent carbolic acid solution in from eight to eighteen days; 1.0 per cent required only five hours to four days, while 2 and 4 per cent solutions required less than six hours.

Neither man, the common experimental animals, nor insects other than honey bees, so far as is known, are susceptible to infection with the European foul brood bacillus.

Methods of Infection and Control.—These are essentially the same as those for American foulbrood.

BACTERIAL SEPTICEMIA OF LARVÆ OF THE LAMELLICORNÆ

Bacillus septicus insectorum—Krassilstschik

HISTORY AND DISTRIBUTION.—This disease occurred separately and together with graphitosis previously described.

SYMPTOMS.—The septicemia produced in larvæ of the Lamellicornæ by *B. septicus insectorum* is characterized by a uniform browning of the body of the larvæ. As death approaches, the larva shrivels up and when dead is about half its natural size and is of a deep brown color. During the progress of the disease, the larva ejects a very black, abundant, viscous, semifluid substance from the anus which soils the extremity of the abdomen.

CAUSAL ORGANISM.—This bacillus is 1.2μ to 1.8μ long and from 0.6μ to 0.9μ in diameter; often in pairs; long filaments not formed. Spores are characteristically dip lospores although isolated ones occur not infrequently. It shrinks very little when stained by Gram's method.

Gelatin is liquefied; subsurface colonies are decidedly lemon shaped, yellowish brown, finely granular, surface of colony typically curled. Surface colonies are concentrically three-ringed, the interior opaque, the second ring more transparent, the third very thin and finely granular. Saccate liquefaction in gelatin stab; the gelatin is blackened and has a very disagreeable odor. Spores are found in the sediment at the bottom of the tube. Broth is made turbid in eighteen to twenty hours, no pellicle, bad odor.

METHODS OF INFECTION.—Healthy larvæ inoculated with *B. septicus insectorum* by placing cotton saturated with a broth culture on a wound, died in most cases with typical symptoms.

The sole habitat of these microbes before death is in the blood system.

BACTERIAL DISEASE OF THE GUT-EPITHELIUM OF *Arenicola ecaudata*, the Lug-Worm

Bacterium arenicolæ.—Fantham and Porter.*

HISTORY AND DISTRIBUTION.—This bacillus was found in the lumen of the gut and within the intestinal epithelium of specimens of *Arenicola ecaudata* obtained from Plymouth, England. This disease is not of frequent occurrence.

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* Fantham, F. B. and Porter, A. *Bacillus arenicolæ*, n. sp., a pathogenic bacterium from the gut-epithelium of *Arenicola ecaudata*. Cent. f. Bakt. I., Orig. 52, 1909, 329–334.

SYMPTOMS.—No external symptoms are noted. Lesions are produced in the epithelium, the cells undergoing degeneration, perhaps shortening the life of the lug-worm. *Bact. arenicolæ* seems to be confined chiefly to the ciliated tracts, as was determined by microscopical examination of sections.

METHODS OF INFECTION.—No methods of infection are noted. From the type of the disease, however, infection *per os* is suggested.

CAUSAL ORGANISM.—*Bacterium arenicolæ* averages about 11μ long and 1μ broad. Extreme individuals measure 7μ to 17μ long by 0.7μ to 1.3μ broad; some of the larger forms are slightly sinuous in outline; no flagella; chromatophile granules determined by staining with iron-hematoxylin, are present, often in considerable numbers, scattered through the cell; these granules are often concentrated into transverse bars both of which in some specimens are refractile. The cytoplasm stains with difficulty with plasma stains. Division is transverse. One terminal spore is formed which does not cause the enlargement of the rod to any extent. No cultural characteristics are given.

IMPORTANCE.—This disease is of no special economic importance.

PSEUDOGRASSERIE OF THE GIPSY-MOTH CATERPILLAR

Bacillus lymantricola adiposus—Paillot

HISTORY AND DISTRIBUTION.—In August, 1917, Paillot (France) noted a gipsy moth caterpillar which presented exterior symptoms of both grasserie and flacherie. It was infected by two coccobacilli to which he gave the names *Bacillus lymantricola adiposus* and *Bacillus lymantriæ β*. The former bacillus was the sole cause of the disease in question as was proved by experimentation. The following season was so dry that epizoötics were rare among insects and no new cases were observed.

SYMPTOMS.—A few hours after inoculation with *B. lymantricola adiposus* the blood of the caterpillar possessed the same milky appearance as that of caterpillars affected with grasserie. This is due, not to polyhedral bodies as in true grasserie, but to the presence of fat globules in the blood. The name pseudograsserie is given to this disease as it resembles grasserie as to external symptoms only.

CAUSAL ORGANISM.—*B. lymantricola adiposus* is a coccobacillus 1μ in diameter and 2μ long; strains well, showing bi-polar staining. In the blood of the caterpillars of *Lymantria dispar*, this organism shows most unexpected forms; e.g., giant forms, made up of a more or less round mass whose diameter may be 7 to 8μ, and greatly elongated bacillus forms, facing or more or less near this mass, of variable lengths (cells 30 to 40μ long have been observed); the round masses have also been observed without the elongated bacillus forms. These giant forms, true forms of growth, are only met with during the earlier period of infection; they disorganize rapidly and give rise to many coccobacilli. At the beginning of their formation the giant bacilli are slowly motile but as they continue to grow larger motility is lost. The normal cells (coccobacilli), however, are very motile. The cultural characteristics are as follows: in ordinary broth, abundant and rapid growth occurs at 37°, slight sediment after two days, no pellicle. Upon nutrient agar the colonies are yellowish white, large, round, and somewhat raised. Saccate liquefaction. Abundant growth on serum with rapid digestion from the second day. Milk is coagulated the third day at 18–20°, no digestion of casein. The following carbohydrates are fermented, glucose, levulose, lactose, saccharose, mannit, maltose, galactose, dulcit, and arabinose. Litmus carbohydrate media are more or less decolorized with the exception of those containing glucose and saccharose.

PATHOLOGY.—The pathogenic action of *B. lymantricola adiposa* manifests itself principally by the disorganization of adipose tissue. About the fifth hour after inoculation the fat globules some of which are yet contained in fat cells, begin to appear in the blood; the proportion of these globules increases rapidly until the bloob becomes milky. This action upon adipose tissue is the first authentic example of this type of specificity among microbial parasites of insects, yet this specificity is only very relative since this organism also multiplies abundantly in the blood.

PATHOGENICITY FOR OTHER INSECTS.—The larvæ of *Vanessa urticæ*, of the brown tail moth *Euproctis chrysorrhea*, and the silk worm show the same symptoms upon inoculation as do the caterpillars os *Lymantria dispar*.

SAC BROOD, A DISEASE OF BEES

Filtrable virus—White*

HISTORY AND DISTRIBUTION.—A disease which was similar to, but was not foul brood was noted in 1881 by Doolittle in America, by Jones of Canada in 1883, and by Simmins of England in 1887. The larvæ were found to die here and there throughout the brood comb; the disease would disappear entirely or it would reappear the next season; the bees would frequently remove the dead brood and no further trouble would ensue. Simmins found no microscopic evidence of disease in these larvæ. In 1892 an editorial in one of the bee journals stated that dead brood had been encountered which did not seem to be infectious and which lacked two decisive symptoms of the real foul brood, *i.e.*, the ropiness and the "glue-pot" odor. In 1902 G. F. White of the U. S. Dept. of Agriculture began the study of this diseased brood. This disease was described in Switzerland in 1906 and later in 1910. It occurs among bees in localities having as wide a range of climatic conditions at least, as are found in the United States.

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* White, G. F. Sacbrood. Bull. 431, B. of Ent., U. S. Dept. Agr., 1917.

The name "sac brood" comes from the fact that many larvæ dead of this disease can be removed from the cell without rupturing their body wall. When thus removed they have the appearance of a small enclosed sac.

SYMPTOMS.—The strength of a colony in which sac brood is present is frequently not noticeably diminished. When the brood is badly infected, however, the colony naturally becomes appreciably weakened thereby. The death of the worker larvæ is the primary cause for the weakness resulting from the disease in a colony. The colony is also weakened by the dead sacbrood larvæ remaining in the cells for weeks as they not infrequently do, thus reducing the capacity of the brood nest for brood rearing. The brood dies after the time of capping. The dead larvæ are, therefore, almost always found extended lengthwise in the cell and lying with the dorsal side against the lower wall. It is not unusual to find many larvæ dead of this disease in uncapped cells. Such brood, however, had been uncapped by the bees after it died. In this disease the cappings are frequently punctured by the bees. Occasionally a capping has a hole through it, indicating that the capping itself had never been completed. A larva dead of this disease loses its normal color and assumes at first a slightly yellowish tint. "Brown" is the most characteristic appearance assumed by the larva during its decay. Various shades are observed. The term "gray" might sometimes appropriately be used to designate it. The form of the larva dead of this disease changes much less than it does in foul brood. The body wall is not easily broken, as a rule. On this account, often the entire larva can be removed from the cell intact. The content of this saclike larva is more or less watery. The head end is usually turned markedly upward. The dried larva or scale is easily removed from the lower side wall. There is practically no odor to the brood combs. Adult bees are not susceptible to the disease.

Causal Organism.—No microörganisms have been found either culturally or microscopically. However, experimental evidence shows that the etiological factor is a filtrable virus. The virus contained in a single larva recently dead of the disease has been found sufficient to produce infection in and death of at least 3000 larvæ within a week. If the virus from one larva each succeeding time were given the opportunity of increasing 3,000-fold, in less than two weeks, theoretically, a sufficient amount of virus would be produced to infect 9,000,000 colonies, more colonies probably than are to be found at present in the United States, and within three weeks enough virus could be produced to inoculate every colony in existence. These figures give an idea of the enormous rapidity with which the sacbrood virus is capable of increasing. However, it is possible that so small an amount of virus may be taken up by an individual larva that no disease results.

The sacbrood virus is influenced by various physical, chemical and biological agencies, which influences, if applied rightly, may constitute control measures. The virus is killed by heating in water at 60° for ten minutes. In honey, however, it is necessary to employ 70° for ten minutes, while if no heat is applied and the virus is shielded from the sun it remains alive nearly a month. Drying at room temperature for approximately three weeks was not destructive but for longer periods of time it was fatal. Dried virus exposed to the direct rays of the sun was destroyed in from four to seven hours; when suspended in water from four to six hours only was required for destruction, but when suspended in honey fatal effects were obta ned on exposure to sunlight for five to six hours. Five days was required to destroy the virus under the influence of fermentative processes in a 10 per cent sugar solution at room temperature, and also in a 20 per cent honey solution at outdoor (summer) temperature. Putrefactive processes (infected larvæ crushed and mixed with soil in water) allowed the virus to remain active for approximately ten days. Carbolic acid in 0.5, 1.0 and 2.0 per cent solution was resisted by the virus for more than three weeks; 4.0 per cent is more effective. However, experiments show that neither this chemical nor quinine should be relied upon as a means of treating sacbrood.

Methods of Infection and Control.—The transmission of any brood disease takes place (1) from diseased to healthy brood within a colony and (2) from a diseased colony to a healthy one. As has been shown experimentally the virus of sacbrood produces the disease in all cases when larvæ, sick and dead of this disease are picked from the combs, crushed, diluted with sterile water, the suspension filtered by means of a Berkefeld filter and the filtrate so obtained fed to healthy colonies either directly or mixed with sirup. From this fact it is fair to assume that sacbrood may result whenever the food or water used by the bees contains the living virus of the disease.

The period from time of inoculation to the appearance of the first symptoms of the disease—the incubation period—is approximately six days, being frequently slightly less. By inoculation the disease may be produced at any season of the year that brood is being reared. Under natural conditions, however, the disease is more often encountered during the first half of the brood-rearing season than during the second half. The course of the disease is not greatly affected by the character or quality of the food obtained and used by the bees.

Bees have a tendency to remove diseased or dead larvæ from the cells. When the removal is attempted about the time of death it is done piece-meal. Just what becomes of these bits of diseased tissue, is not known. If these fragments were fed to young healthy larvæ within a week, they would most likely become infected with sacbrood. Experience, however, shows that under these conditions the tendency in a colony is in most cases toward recovery. This suggests that the workers may feed the infected tissues to the older larvæ or to adult bees; in either case the likelihood of the transmission of the disease would apparently be very materially reduced. If the infective material were stored with the honey and did not reach the brood in a month or six weeks, again it is not probable that the disease would be transmitted.

If the infective material is removed from the hive and freed from the adult bees removing it, experimental evidence indicates that during the warmer seasons at least, there is but little chance of the virus being returned to the hive and producing any noticeable infection.

There is also little probability of the virus of sacbrood being transmitted by way of flowers visited by bees. There is, however, a greater likelihood of the water supply being a source of infection.

Bees drifting or straying from infected colonies to healthy ones have been proved to be less liable to transmit the disease than when robbing occurs. It is not yet known in what way the sacbrood virus is carried over from one brood rearing season to another.

From knowledge obtained through experimentation but few control measures can be advocated. Most of the advice which can be given is negative. Theoretically it is better to store combs from sacbrood colonies for one or two months before they are again used, as drying apparently destroys the virulence of the virus within this time. After the early brood-rearing season of the year is past brood frames from badly-infected colonies may be inserted into strong, healthy ones, and cause thereby very little infection and consequently only a slight loss. This is the preferable method of disposing of the infected combs for the practical beekeeper to employ. In practical apiculture no fear need be entertained that sacbrood will be transmitted by the hands or clothing of the operator, by the tools used about the apiary, through the medium of the wind, or by the queen. Flaming or burning the inside of the hive, or treating the ground about a hive containing a sacbrood infected colony appears to be entirely unnecessary.

WILT DISEASE OR FLACHERIE OF THE GIPSY MOTH CATERPILLAR,

Porthetria dispar L.

Filtrable virus—Glaser*

HISTORY AND DISTRIBUTION.—There is no account of the occurrence of wilt in America prior to 1900. This disease may have been introduced on trees or shrubs imported from Europe, in which country "Wipfelkrankheit," a wilt disease of the European nun-moth caterpillars, *Psilura monacha*, exists. In Europe flacherie has become the "guardian angel" of the central European forests.

In the United States there is every reason to suppose that the wilt is distributed over the entire territory infested by the gipsy moth, a territory of about 4,850 square miles (1915) extending over various parts of Maine, New Hampshire, Massachusetts and Rhode Island.

SYMPTOMS.—The symptoms of the wilt disease of the gipsy moth caterpillars are those of flacherie of the silk-worm. They soon stop eating, become languid, usually crawl up on some object where they remain motionless. In a few hours there drops from the mouth and anus a dirty, blackish, foul-smelling liquid; they become more and more flaccid, one leg after another looses its support and finally the caterpillar reduced to a black skin is found hanging limply to tree trunks and limbs, still holding on with one or two of its false feet or with the anal claspers. After death their body tissues become degenerated so rapidly that it is impossible to handle them; a slight touch breaks the skin and a thin dark, offensive-smelling liquid flows out, consequently they can never be used for histological work.

CAUSAL ORGANISM.—A filtrable virus seems to be responsible for the death of these caterpillars It is filtered with difficulty, however Bacteria are not responsible for this disease. Minute dancing granules are observed in the Berkefeld filtrate, which may be etiologically significant. No bacteria or polyhedral bodies are observed in the filtrate.

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* Glaser, R. W. and Chapman, J. W. The Wilt Disease of the Gipsy Moth Caterpillar. Jour. Econ. Entomol., 6, 1913, pp. 479–488.

Reiff, W. The Wilt Disease, or Flacherie, of the Gypsy Moth, 1911.

Methods of Infection.—Infection naturally takes place through the mouth by means of the food. Predisposition to the disease is secured by giving the caterpillars food which has been placed in water and renewed only every three or four days. This causes an increase in the acidity of the leaves which in turn decreases the alkalinity of the caterpillar's digestive fluid. Before the visible outbreak of flacherie, as an early symptom, a characteristic sweet odor is recognized in the breeding cages which resembles that of withered lilac blossoms somewhat. Whenever this odor is noticeable, flacherie soon makes its appearance, and as it progresses the odor increases proportionately (Fischer).

Lack of food, which is necessarily brought about by the caterpillars themselves, causes them to lose their vitality, thus producing a greater susceptibility to the disease. Defoliation also exposes them to the sun's rays which have the effect of converting the chronic into the acute form of wilt. In lightly infested woodland this does not happen as the caterpillars can always find shade. Flacherie, however, seems to be influenced by climate and weather conditions less than any other caterpillar disease.

Wilt is always prevalent among the older caterpillars; young caterpillars often live several days before succumbing to the disease. Female caterpillars always succumb more readily to the wilt disease than the male; this may perhaps be due to the fact that they require a longer time to mature than the male. Diseased females deposit egg clusters reduced in size, which contain usually, embryos, incompletely or not at all developed. In this case there are always found undeposited eggs within the body of the female, which never occurs with healthy moths. Genetic immunity of certain individuals is probable. Sublethal doses of the virulent filtrate may produce active immunization. Although probable, there is yet no definite evidence that wilt is transmitted from one generation to another.

Pathology of Wilt.—When a caterpillar dies of wilt, all of its tissues are in a state of disintegration. The intestine is the last internal organ to disintegrate. A smear of the brown liquid from a dead caterpillar examined microscopically with a high power lens will be found to contain, besides the elements of disorganized tissues, myriads of highly refractive polyhedral bodies of various sizes. The average polyhedron measures from 1μ to 6μ in diameter and is never regular as are the silk-worm polyhedra. The significance of these bodies is not known. However, they are believed to be reaction bodies belonging to the highly differentiated albumins, the nucleoproteids. They may be stages of the filtrable virus but no evidence has been brought forward to substantiate this view. It has been determined however, that no diagnosis of wilt is valid unless polyhedra are demonstrated microscopically.

The "Wipfelkrankheit" of the nun-moth in Germany is essentially the same disease as that of the gipsy moth in the United States (Escherich and Miyajima.)

PÉBRINE, AN INFECTIOUS DISEASE OF THE SILK-WORM, *Bombyx mori*

Nosema bombycis

HISTORY AND DISTRIBUTION.—About the year 1853, anxious attention began to be given in the southern part of France to the ravages of a disease among silk-worms which from its alarming progress,threatened to issue in national disaster. Symptoms of this disease had been noted as early as 1845. It finally became necessary to import seed (technical term for eggs) for continuing the culture of the silk-worms. This was procured first from Lombardy, but after one successful year the same disappointments occurred. Then Italy was attacked, also Spain and Austria; later seed was procured from Greece, Turkey, the Caucasus, but to no avail; China itself was attacked and in 1864, healthy seed could be obtained only from Japan.

This disease, characterized by dark spots on the silk-worms, was called pébrine, from the patois word pébré (pepper), the name given to it by de Quatrefages on account of the resemblance of these spots to pepper grains.

SYMPTOMS.—As above mentioned, one of the symptoms of pébrine is the manifestation of dark spots in the skin of the larvæ; some worms languish on the frames in their earliest days, others in the second stage only, some pass through the third and fourth molts, climb the twig and spin their cocoons. The chrysalis becomes a moth, but the moth shows signs of disease in its deformed antennæ and withered legs; the wings seem singed. Eggs from these moths are inevitably unsuccessful the following year. Thus, in the same nursery in the course of the two months that it takes a larva to become a moth, the pébrine disease is alternately sudden or insidious; it bursts out or disappears, it hides itself within the chrysalis and reappears in the moth or the eggs of a moth which has seemed sound.

Causal Organism.—The causal organism for this disease is *Nosema bombycis*, a protozoön belonging to the *Microsporidia*. The spores find their way from the caterpillar by means of the dejecta or through the disintegration of dead forms to other silk worms. Some of the parasites find their way into the ovary, produce spores, pass through the pupal and imaginal stages of the host into the next generation of silk-worms. The spores are often regarded as pébrine corpuscles

In the worms suffering from pébrine, corpuscles or polyhedral bodies, first noted by Pasteur, are found in all tissues and all fluids of the body, even in the material from which the silk is made; naturally they are also found in the dejecta of the worms. These same bodies are found in and on the infected eggs, pupæ and moths and in innumerable quantities in the dust of the infected nurseries; they are easily recognized microscopically.

These polyhedral bodies are now known not to be the etiological factor, but are most probably an effect of the disease. Glaser and Chapman* have found them to be nucleo-protein crystal-like degeneration products of the insect blood cells, and not organisms. They contain iron and phosphorus. Crystals simulating the original polyhedra are obtained on dissolving polyhedra in alkali dialyzing out the alkali, and evaporating the protein solution. Before their nature was known, however, elimination of all eggs containing these bodies resulted in the suppression of the disease.

Methods of Infection.—A very common method of infection is due to the habits of the silk-worms crawling over one another. When a worm moves across a diseased worm, its claws cut through the tegument and become contaminated; in its progress it inoculates other worms by means of its soiled fangs. The greatest source of contagion, however, is the excreta which fall on the food of the worms. Luckily this infectious material on being exposed to light and air, becomes rapidly attenuated. However, the causal organism is not so attenuated when within the egg; it passes the winter in a latent state and develops along with the worm, multiplying within its body and altering more or less profoundly the conditions of existence.

Control.—If moths are not seriously diseased, their eggs will always furnish several healthy larvæ and if these are isolated as soon as they hatch out and are kept and bred under sanitary conditions, a race of worms free from corpuscles can soon be obtained. This has been found to be the most effective method of combating pébrine. Excessive heat saps the vitality of the silk-worm and makes it ready prey to disease. Open air cages result in much hardier, more active worms.

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*Glaser, R. W. and Chapman, J. W. The nature of the polyhedral bodies found in insects. Biol. Bul. Marine Biol. Lab. Woods Hole, Mass., 30, pp. 367–390, 1916.

Nosema-Disease of Bees

Nosema apis—Zander

History and Distribution.—This disease was apparently first noticed about sixty years ago by Dönhoff (1857) who discovered, microscopically, small oval bodies in the stomachs of adult bees which he supposed had died of exposure. He sent some of the bees to Leuckhart who after examining them microscopically expressed the belief that the oval bodies were the spores of a fungus. So Dönhoff referred to this bee disorder as "Pilzsucht" (fungous disease). In 1909 Zander found similar small oval bodies in the walls of stomachs taken from affected bees and to these organisms he gave the name, "*Nosema apis*" and for the disease he (1911) used the name "Nosemaseuche." This disease is known in Europe under similar names, "Nosemakrankheit" in Switzerland (Nussbaumer, 1912; Angst, 1913), "Nosemasygdommen" in Denmark (Bahr, 1915). Nosema infection has been reported also from Australia (1910), Brazil (1911), Canada (1914), England (1911), and Germany (1909). White* has found Nosema-disease in samples of bees received from 27 different states of the United States. The distribution in the United States, however, has been hard to determine as beekeepers have not learned to recognize the disease produced by *Nosema apis* by any one name. "Spring dwindling" and "weakened colonies" were descriptive terms applied by beekeepers to colonies in which *Nosema apis* was found. The highest percentage of Nosema-infected bees occurred in weak colonies.

Symptoms.—Nosema-disease is an infectious disease of adult honey-bees. It is not particularly malignant in character, being more in this respect like sacbrood than the foulbroods. So far as is known, all races of honey-bees are susceptible. This disease presents only a few symptoms. These White described as characteristic of the colony rather than of the individual as a unit, since it is the colony as a whole that is of primary interest to beekeepers.

*White, G. F. Nosema-disease, Bul. 780, U. S. D. A. 1919, pp. 59.

Weakness is a colony symptom which will invariably be manifest if a sufficiently large percentage of the bees of the colony are Nosema-infected and if the infection persists for a sufficient period. When only a small percentage of the bees are infected the weakness resulting may never be apparent. The loss in strength may be gradual or sudden.

A Nosema-infected colony behaves similarly to a healthy one. The stores are sufficient. The queen does her work well. As the colony dwindles the queen is usually among the last handful of bees. The brood in general is normal in appearance, but in colonies weakened by the disease not infrequently it is seemingly in excess of the amount that can be properly cared for by the adult bees present. The workers, especially the young ones, are most frequently infected, although drones and queens are susceptible. It is not unusual to find from ten to twenty per cent of the workers of diseased colonies infected. An infected bee manifests no outward symptoms of the disease when seen among the other bees of the colony and it performs functions similar to those performed by healthy ones.

When the stomach of an infected bee is removed it may show marked changes which are characteristic of Nosema-disease. The brownish yellow or dark reddish hue of the normal stomach becomes gradually lost as the disease advances. The organ is often increased in size, the constrictions are less marked, and the transparency is diminished. In late stages of the disease, however, the stomach approaches the normal in size and the constrictions are again well marked. The organ is then white and opaque and the tissues are fraible and easily crushed. When crushed the mass presents a milky appearance.

Upon microscopic examination, *Nosema apis* is found in very large numbers in the crushed tissues. The presence of the parasite is almost invariably recognized by its spore form. The presence of Nosema-infected bees in a colony is the one constant colony symptom of the disease.

Causal Organism.—White proves that *Nosema apis* is the cause of Nosema-disease by a process of elimination. Malden (1912–1913) who studied the bacteriology of Nosema-infected bees found that although the number of bacteria in diseased bees, was much greater than in normal ones, there was no evidence of a direct etiological relation existing between these bacteria and the disease. White himself found that Nosema-disease is not caused by a filtrable virus. Higher animal parasites and fungi being absent, and the bacteria and the filtrable viruses thus being eliminated, tentatively at least, there remains only one group, the protozoa, and of this group there is only one species, *Nosema apis*, that is constantly present in Nosema-disease. The conclusion is naturally reached, therefore, that *Nosema apis* is the cause of Nosema-disease. Such a conclusion is in harmony with views generally accepted at the present time in regard to proof necessary to establish the causal relation of such a germ to the disease.

The spore form of *Nosema apis* is the form most encountered and most readily recognized in making an examination for the parasite. These spores in unstained (India ink) preparations are small, refractile, more or less oval bodies varying somewhat in size, but having an average length of 4.46μ and an average breadth of 2.44μ. In stained preparations the average length and breadth are 4.15μ and 2.06μ respectively. The spore is surrounded by a somewhat resistant coat which tends to maintain for it a constant form, but it is not a rigid structure, since, when studied in fresh preparations it will be seen to bend to and fro as it is carried along by a current under the coverglass. A source of confusion in the diagnosis of *Nosema apis* in adult bees is the fact that starch granules from pollen grains of corn and of most of the cereals closely resemble the spores of *Nosema apis*. This organism has not been cultivated in pure culture by artificial methods.

Nosema apis suspended in water is destroyed by heating for ten minutes at 58°; when suspended in honey, 59° is required for destruction. When dried at room and outdoor temperatures respectively it remained virulent for about two months, at incubator temperature about three weeks, and in a refrigerator about seven and one-half months. When dry, fifteen to thirty-two hours direct exposure to the sun's rays is necessary for total destruction; when suspended in water thirty-seven to fifty-one hours is required, while if suspended in honey, destruction is frequent as the temperature of the honey reaches or exceeds 60°, a temperature at which the germ is killed by heat. If placed in honey and shielded from the light *Nosema apis* remains virulent for two to four months at room temperature. Fermentative processes are destructive to the spores in a 20 per cent honey solution in three days at incubator temperature, and in nine days at outdoor temperature while in a 10 per cent sugar solution it is destroyed in from seven to eleven days at room temperature. When subjected to putrefactive processes (suspensions made in a 1 per cent aqueous peptone solution) *Nosema apis* resists destruction for five days at incubator temperature, for two weeks at room temperature, and for more than three weeks at outdoor temperature. *Nosema apis* spores in the bodies of dead bees cease to be virulent in one week at incubator temperature, in four weeks at room temperature, in six weeks at outdoor temperature and in four months in a refrigerator, while if the bodies of the dead bees are lying on the soil virulence ceases in from forty-four to seventy-one days. A 1 per cent solution of carbolic acid destroys the spores of this organism in less than ten minutes.

METHODS OF INFECTION.—In general, the manner in which a bee becomes infected with *Nosema apis* is as follows: Spores which have left the body of an infected bee with the excrement are ingested by the healthy adult bee. As the excrement is usually voided in flight this influences the chance of infection. The environment within the stomach of the bee is favorable for the growth and multiplication of the parasite. The digestive fluids are believed to assist in removing the spore coat. The liberated young parasite finds its way to the walls of the stomach and invades the epithelial cells. Within this epithelial tissue it grows and multiplies with great rapidity, giving rise finally to numerous spores. The cells of the epithelium at times seem to become virtually filled with the parasites. That portion of an epithelial cell that is normally shed into the lumen of the stomach in case of infection bears with it many spores. These are liberated gradually from the fragments, become mixed with the partially digested food in the stomach, and are carried onward first into the small and then into the large intestines, and finally pass out of the alimentary tract with the excrement. Other bees ingesting these spores become infected. This, in brief, is the life cycle through which the parasite passes.

In infecting the stomach the parasite reaches the basement membrane but does not penetrate it. The muscular part of the organ is, therefore, uninvolved. Likewise when found in the Malpighian tubules the infection does not proceed beyond the basement membrane. The protozoön does not infect the pharynx, the œsophagus, the honey sac, the proventriculus, the small or the large intestine—organs which possess a pronounced chitinized intima. So far, *Nosema apis* has not been encountered in the blood, musculature or any of the other tissues of the body.

Infection in apiaries has been found to occur at all seasons of the year but is greatest during the spring. Experimentally, however, it has been found that bees are susceptible to *Nosema apis* infection the year round. The rôle played by food in the causation of Nosema-disease is slight, if indeed it contributes at all appreciably to it.

The fact determined experimentally, that a suspension of *Nosema apis* in syrup when fed to bees will produce the disease, shows quite conclusively, however, that infection takes place through the ingestion of the parasite. At present there is no evidence that it takes place otherwise than by way of the alimentary tract. The facts which are known concerning Nosema-disease indicate that the disease may be transmitted: (1) From the infected bees of a colony to healthy bees of the same colony, and (2) from the infected bees of a colony to healthy bees of another colony. Under certain circumstances the infection is not readily transmitted within the hive. For example, colonies which in the spring of the year show less than 50 per cent of Nosema-infected bees are likely to recover from the infection without treatment. A colony may contain a small percentage of Nosema-infected bees throughout the year and not become heavily infected at any time. Colonies experimentally inoculated in June, July, or August are practically free from the infection within six weeks. This is probably due to the fact that the young bees replace those dying of the infection.

To the contrary, however, when heavy losses occur among the workers in the spring the colony suffers as there are no young bees to replace those lost as a result of the infection.

Colonies which die out or become weakened by the disease furnish conditions which invite robbing. Robbing in a certain number of cases probably results in the transmission of the disease but this likelihood seems not to be nearly as great with Nosema-disease as in the case of the foulbroods.

Death from Nosema-infection takes place during the active bee season in from two weeks to a month; during winter the disease may run two or three months or even more. Infected drones die sooner than infected workers but infected queens probably live longer. It is quite likely that the age of the bee when infected is not a negligible factor in determining the course of the disease. Whether a bee once infected ever recovers from the infection has not yet been established definitely. From what is known of diseases in man and animals, however, recovery might be expected in a certain percentage of Nosema-infected bees. The data so far indicate that recovery from Nosema-infection among worker bees is comparatively rare.

METHOD OF CONTROL.—Experiments show that brood combs need not be destroyed as no Nosema-infection occurs even when brood-combs from Nosema-infected colonies are inserted immediately into healthy colonies. When medicated diluted honey is fed to Nosema-infected colonies, using different drugs, some drugs prove efficaceous while others have no effect. White states that these latter experiments are altogether too few for definite conclusions as to the extent of their action.

Two probable sources of infection are the presence of a sluggish body of water near an apiary which is used by bees as a water supply and the robbing of diseased colonies, and these are more or less under control. The disease does not seem to be spread through the medium of flowers, by the hands and clothing of the apiarist, the tools used about an apiary, winds, hives which have housed infected colonies, combs from such colonies, or bees dead of the disease unless they serve to contaminate the water supply. Thus these latter mentioned improbabilities can be eliminated when dealing with control measures.

MISCELLANEOUS INSECT DISEASES

ENTOMOPHTHORACEÆ*

ENTOMOGENOUS FUNGI.*—*Empusa muscæ* is the best known member of this group of insect parasites. This species attacks the house fly. The fly weakened by the disease fastens itself to the wall or window pane by its mouth parts. The mold fruiting upon the dead body discharges its spore masses which adhere where they strike. Such dead flies are frequently surrounded by a discolored circle where great numbers of these spores are fixed upon the wall. Natural epidemics of this kind frequently destroy flies and other insects but attempts to produce such epidemics by inoculation have generally failed because the fungus is too greatly dependent upon uncontrollable factors of temperature and humidity.

Other entomogenous fungi include representatives of many different groups. The life histories of some of them are well-known but many of them have been only partially studied. The practical usefulness of some of these species, notably *Sporotrichum globuliferum*, as a chinch-bug disease, has been studied carefully. While the work was markedly successful in causing an epidemic disease when conditions favored it, dependence upon particular conditions was so complete that the production of the disease as an effective destroyer of pests failed. Similar results have attended the effort to use other fungi as insect-destroyers. The conditions which make possible their development in epidemic form only occur occasionally. These conditions in themselves are, as a rule, very unfavorable to insects. Under other climatic conditions, these diseases appear only as isolated cases, negligible in their effect upon the insect population, no matter how carefully the inoculating material is spread by man.

One great series of fungi the Laboulbeniales is limited to insect parasitism. The genera and species of this group are very specialized forms producing local lesions and typical fruit bodies. Thaxter has described and figured a great number of these species.

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* Prepared by Charles Thom.

Other Microbial Diseases*

A new saccharomycete, *Monosporella unicuspidata*, n.g. and n.sp., was found by Keilin to be parasitic in the body cavity of the ceratopognoid larva (*Dasyhelea obscura*) which usually lives in the thick brown sap that fills the infected wounds of elm or horse chestnut.

Paillot describes five species of coccobacilli which infect the caterpillar of *Pieris brassicæ*, namely, *B. pieris fluorescens*, *B. pieris liquefaciens*, *B. pieris non-liquefaciens α* and *B. non-liquefaciens β* and *B. pieris agilis*.

Three new bacteria parasitic in the caterpillar of the gipsy-moth have also been described by Paillot. One of these resembles *B. lymantriæ* of Picard and Blanc, the other two are *Diplococcus lymantriæ*, a species quite different from that of Hanneton, which causes a very energetic phagocytosis to take place in the blood of the insect but is not very pathogenic, and *Bacillus liparis* which resembles *Bact. diphtheriæ* morphologically. This last named organism also induces a very active phagocytosis, but is more pathogenic than *D. lymantriæ*.

A silk worm disease in Japan is produced by a spore-forming organism *Bacillus sottō* ("Sottō" is a Japanese term signifying "sudden decay"), the ill effects of which are due to a toxin which largely remains fixed in the organism and in the spores. Atoxogenic strains of this bacillus occur which can be distinguished from toxogenic strains neither culturally nor through immunization. (Aoki and Chigasaki).

Paillot has found the cockchafer to be susceptible to many varieties and races of coccobacilli which cause septicemias. Four types of *B. melolonthæ* are found, one a liquefying type, the other three nonliquefying. In about 30 per cent of the septicemias caused by *B. melolonthæ* a secondary infection occurs. Three different associated diseases have been studied, due (1) to *B. melolonthæ nonliquefaciens β* and a Gram-positive diplococcus, *Diplococcus melolonthæ*, (2) to *B. melolonthæ liquefaciens* and *Diplococcus melolonthae*, and (3) to *B. melolonthæ liquefaciens* and a large sporulating bacillus, *B. hoplosternus*. *B. hoplosternus* is very pathogenic for the cockchafer and the caterpillars of *Vanessa urticæ*, *Euproctis chrysorrhea*, *Chelonia caa,j*

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* Prepared by Z. Northrup Wyant.

Malacosoma neustria, *Hanneton commun* and *Hanneton de la Saint-Jean* (*Rhyzotrogus solsticialis*), but does not kill the gipsy moth caterpillar, *Lymantria dispar*, regularly, even after many passages.

Serbinov describes an infectious diarrhœa of bees on the south coast of the gulf of Finland due to two new bacteria, *Bact. coli apium* n.sp. and *Proteus alveicola* n. sp. The disease develops rapidly and becomes epizoötic, the bees become weakened and death follows, frequently in convulsions.

Korke of India describes a new protozoön, *Nosema pulicis* n. sp. which has been found to cause frequent infections of the digestive tract of the dog flea, *Ctenocephalus felis*. This parasite was infected so readily that in about three weeks the infection rose from about 16 per cent to nearly 100 per cent under controlled conditions.

Bacillus erausquinii n. sp. was isolated from locusts of the species *Romalea miles* in Argentina by Cullen and Maggio. It is said to have many characteristics which distinguish it from *B. acridiorum*.

A disease of the caterpillars of *Gortyna ochracea*, an artichoke pest, is recorded in the Department of Var, France. *Bacillus gortynæ* was isolated as the causal factor.

Bacillus pyrameis I and II were isolated from the blood and tissues of the caterpillars of *Pyrameis cardui*, another artichoke pest. These may be distinct or merely varieties of a single species; they may represent one or more saprophytic species widespread in nature which are readily adaptable to a parasitic life (Paillot).

Two associated microörganisms, one a motile rod and the other a coccus were the cause of epizoötics destroying nearly all of the caterpillars of *Galleria melonella*, the bee moth, which were being raised for experimental purposes (Metalnikov). The rod form was the more virulent on injection. The manner in which infection takes place was not determined.

General Pathology and Immunity Studies

The study of degenerative changes in normal and in pathological blood cells of insects is especially important as the blood is frequently used in diagnosing the health of a particular insect. Glaser* (1917) succeeded not only in growing normal insect blood cells *in vitro* but in observing *in vitro* the formation of typical polyhedra from the blood of healthy *M. americanum* and *Porthetria dispar* larvæ fed with filtered polyhedral virus (Berkefeld "N" filter used). He found that it was impossible to infect the blood directly with the virus. The virus had to be given "a start" within the insect itself. Later stages of the virus however, find the conditions suitable on the tissue culture slides.

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* Glaser, R. W. The growth of insect blood cells *in vitro*. Psyche 24, 1917. pp. 1–6, 1 pl.

Glaser observed from his studies that normal blood cells have a normal tendency towards crystalline disintegration, thus it is not surprising that crystals (polyhedra) are found within the degenerating nuclei in a series of insect diseases.

The ability of insect tissue to grow well seems to vary to a slight degree according to the species of the insect.

Glaser (1918) in studying immunity principles in insects found that while entomological text-books emphasize the importance of phagocytosis by blood cells called amebocytes, in ridding the insect body of foreign matter, in reality these insect blood cells are visibly rather passive. Experiments on various insects with bacteria pathogenic for the species, showed that the normal blood did act antagonistically toward the organisms introduced, but the riddance was not accomplished by hungry amebocytes; the only movement shown by these blood cells *in vitro* consisted in cell division. The antagonistic substances are extracellular and, therefore, in the blood plasma or serum. Microscopic observation with grasshopper, army worm and gipsy moth caterpillar blood showed that when apparent phagocytosis occurred the infecting bacteria seemed to bore their way into the cytoplasm. This may have been due to surface tension, however, and might be called phagocytosis if the word is used in a broad sense. On the culture slides, the quantity of the blood is not sufficient and metabolism is lowered, so that antagonistic substances are not formed so rapidly nor so abundantly as is the case within the body of the insect.

The blood of several grasshoppers (*Melanoplus femur-rubrum*) which had been immunized to *Bacillus poncei* by the injection of 0.1 cc. of a twenty-four hour broth culture was successfully used in demonstrating the presence of a specific agglutinin. The blood of uninfected grasshoppers failed to show this phenomenon. The presence of bactericidal substances in immune insect serum was also proven. Actively immunized grasshopper blood showed a high degree of antagonism toward the bacteria used in producing this immunity. The extent of immunity studies possible with insects seems to be limited by the fact that the insects, grasshoppers, and caterpillars, do not seem to be able to overcome the effects of a second injection.

The agglutination reaction has been successfully applied by Aoki and Chigasaki in the examination of silkworms for differentiating *B. sottō* from *B. megaterium* and *B. alvei* which it resembles, as this reaction is strongly specific for the former. Aoki has also worked with the precipitation reaction using silk worm caterpillar immune serum.

DIVISION X*

MICROBIAL DISEASES OF PLANTS

INTRODUCTION

Although the earliest study of bacterial diseases in plants antedates the isolation of the tubercle bacterium and the cholera spirillum, this branch of bacteriology has not been marked by the progress which has characterized the investigation of animal diseases. The loss of a human life or of a valuable domestic animal has appealed to the student of disease more strongly than the blighting of a pear tree, or the wilting of a potato vine, and, quite naturally, he has directed his efforts along those lines which have offered the greater inducements, and which have demanded immediate attention.

However, with the introduction of new plants, foreign seeds, and strange nursery stock, many previously unheard-of plant diseases have made their appearance. As the farming communities have become more thickly populated, with less uncultivated land between the fields, these diseases have spread from farm to farm more rapidly than in the earlier days, and the losses from these causes have been so heavy during the past decade that the farmers, gardeners and orchardists have come to the Agricultural Experiment Stations all over the country for advice and assistance in combating their troubles. This has stimulated an increased interest in plant diseases, especially along bacteriological lines, with the result that to-day some forty bacterial diseases of plants have been described.

It is a matter of not infrequent observation that closely related species of plants, as well as animals, exhibit a marked difference in their susceptibility to the same disease-producing agents. The Bartlett pear, for example, suffers more severely from blight than the Kieffer, and, among apples, the Toleman Sweet more than the Rome; the small-leaf, stemmy varieties of tobacco seem to be more resistant to the Granville wilt than the large-leaf kinds. Resistance of this sort, which appears to be nothing other than a natural, inborn quality, may be designated as *natural immunity*, and it is immunity of this kind which plant breeding for disease resistance has secured. A good illustration of this is to be found in the wilt-resistant water melon of the Carolinas, which is the result of crossing a naturally susceptible water melon with a naturally resistant citron.

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* Prepared by W. G. Sackett, except a protozoal disease "Fingers and Toes" by J. L. Todd.

Acquired immunity in the plant world is a field yet to be explored. Cases have been cited in which *active immunity* appears to have followed the disease, but these are extremely rare and the evidence is very questionable. *Passive immunity*, at the present time, is unknown.

CHAPTER I

BLIGHTS

STEM BLIGHT OF ALFALFA

Pseudomonas medicaginis—Sackett

HISTORY AND DISTRIBUTION.—The disease has been known in Colorado since 1904 and was described briefly by Paddock in 1906 and more fully by Sackett in 1910. It is distributed generally over Colorado, and is reported to occur in Utah, New Mexico, Arizona, Nevada, Nebraska and Kansas.

SYMPTOMS.—The disease is primarily a stem infection. In the earliest stages, the stems have a watery, semi-transparent, yellowish to olive green appearance along one side. Soon there oozes from the diseased tissue a thick, clear, viscid liquid which spreads over the surface and collects here and there in little bead-like droplets. The exudate also dries in a short time with a glistening finish, and gives the stems very much the appearance of having been varnished, and where the liquid has collected in little amber-colored scales and has hardened, it looks as if the varnish had run and dried. Stems in this condition have a dry, slightly rough feel to the touch. The exudate also dries uniformly over the surface or just beneath it, and there produces a dark brown, resinous surface which blackens with age. Such stems are very brittle and easily broken, which fact makes it almost impossible to handle the crop without an immense amount of shattering The leaves attached to the blighted stems usually show the disease, and sometimes they exhibit the infection independent of the stem. In this case, the petioles become watery and pale yellow, then droop. The malady may be confined to the petiole and base of the leaflet, or it may involve the whole of the blade. Occasionally leaves are found where the inoculation has been made, apparently, in the margin of the leaflet, and the infection has proceeded toward the middle. In such instances, the tender tissue has a watery look, as if it had been bruised.

One-year-old plants may exhibit blackened areas in the crown, and black streaks which run down into the tap root. As the plant grows older, this blackening increases until the whole crown becomes involved, and either the crown buds are destroyed or the root is no longer able to perform its functions, and the plant dies.

So far as our present observations go, the disease appears to run its course with the first cutting, and those plants which have sufficient vitality throw out a good growth for the second and third cuttings.

Cause of the Disease.—If a small piece of the yellowish green, watery tissue from a diseased plant, or a fragment of the dried exudate is placed in a drop of clean water on a glass slide, there will appear on all sides of it, after half a minute, a dense, milky cloud, which can be seen readily with the naked eye, and which slowly diffuses out into the drop. When this preparation is examined under the low power of the microscope, this milky zone easily resolves itself into swarms of motile bacteria.

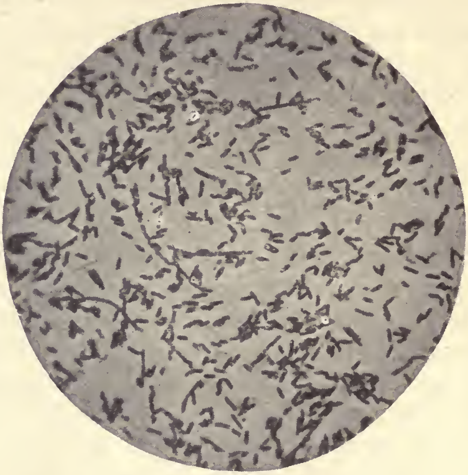

Fig. 195.—*Pseudomonas medicaginis.* Twenty-four hour culture on nutrient agar; stained with aqueous fuchsin; ×1000. (*Original.*)

The organism grows readily upon the ordinary culture media and pure cultures of the germ, inoculated into scarified stems of healthy alfalfa plants, produce the disease in seven to nine days with typical symptoms.

METHOD OF INFECTION.—Under field conditions the causal organism which, presumably, lives in the soil, enters the plants early in the growing season with soil through stems which are cracked and split by late freezing. In some instances, inoculation appears to take place by stomatal and water pore infections.

CAUSAL ORGANISM.—The writer has given the name *Ps. medicaginis* to the causal organism, the characteristics of which are as follows: It is a short rod with rounded ends, about 1.2μ to 2.4μ by 0.5μ to 0.8μ the majority being 2.1μ by 0.7μ. It is actively motile by 1 to 4 bi-polar flagella; non-spore forming and non-capsule forming. Filament formation occurs frequently. The organism stains readily with the aqueous stains, but is Gram-negative.

It produces a surface pellicle on broth. Shining, grayish white on nutrient agar, becomes fluorescent green after three days. Gelatin stab, surface growth only, and no liquefaction. Potato discolored, moderate growth, cream to light orange yellow, starch not destroyed. No growth in Cohn's solution. Good growth in Uschinsky's solution. Plain milk shows no change. Litmus milk becomes bluer after seven days, no curd and no peptonization in thirty days. No indol. No hydrogen sulphide. Ammonia produced from asparagin solution, Dunham's solution and nutrient broth, but not from nitrate broth. Nitrates not reduced. No gas and no acid from dextrose, etc. Obligative aerobe. Optimum temperature 28°; no growth at 37.5°. Thermal death-point 49.0° to 50.0°. Habitat, soil. Pathogenic for alfalfa (*Medicago sativa*).

CONTROL.—The only practical way of combating and controlling the blight is by the introduction of resistant varieties, but no entirely resistant strain has been obtained up to the present time, although the Grimm alfalfa is practically free from it.

As a means of control, the writer recommends that the frosted alfalfa be clipped, as soon as there is reasonable certainty that danger from late frosts is past. This will rid the plants of the diseased portions, and afford an opportunity for the early growth of a new cutting. If this is done in time, the regular number of cuttings should be secured with little or no loss in tonnage.

BACTERIOSIS OF BEANS

Pseudomonas phaseoli—Erw. Smith

Frequently the foliage, stems, and pods of the common beans, as well as the Lima bean are attacked by a bacterial disease.

SYMPTOMS.—The pods and leaves seem to furnish the best food supply for the microörganism, and it is here that we find the most typical lesions developing. Small, reddish spots appear which increase rapidly in size and develop into watery, amber-colored blisters, surrounded by a pink or reddish border. These blisters are filled with myriads of bacteria, and in time, they dry down, forming a pale yellow or amber-colored crust over the affected areas. Ultimately the diseased leaves become brittle, ragged, and are worthless, while the pods curl, shrivel, and rot.

METHOD OF INFECTION.—It is believed that the disease is introduced with the seed, and when once established, is spread from plant to plant by rain, dew, and leaf-eating insects.

CAUSAL ORGANISM.—*Ps. phaseoli* Smith,* is a short, motile rod with rounded ends, which produces a characteristic yellow growth on the different culture media. Gelatin slowly liquefied. Milk becomes slowly alkaline, casein is precipitated by lab ferment and partially redissolved. Very marked diastatic action on potato starch. No gas from glucose, saccharose, etc. Aerobic. Uschinsky's solution, growth feeble and retarded. Thermal death-point 49.5°.

CONTROL.—Care should be taken to select seed from healthy fields where the disease has never occurred. The disease has been partially controlled by spraying with Bordeaux mixture when the plants were 2 to 3 inches high, again ten days later, and after blossoming.

BLIGHT OF LETTUCE

Ps. viridilividum—Brown

The disease has been reported recently from the lettuce-growing sections of Louisiana, and is described as producing a shriveled, dried, burned aspect of the outer leaves, some of which may be in a soft, rotted condition. The deeper leaves exhibit numerous separate or fused spots with a water-soaked appearance; the center of the head is not necessarily involved.

CAUSAL ORGANISM.—Miss Nellie A. Brown† has described the causal organism, *Ps. viridilividum*, as a short rod with rounded ends, motile by 1–3 polar flagella; stains readily with the ordinary stains; is Gram-negative. No spores have been observed. In young agar cultures, the growth is cream-white mottled with yellow, the mottling disappearing with age. Gelatin is liquefied slowly. Nutrient broth is clouded and becomes lime-green in color after ten days. On potato it produces a characteristic transient dark-blue green color which develops promptly and disappears on the sixth day or earlier. Growth develops readily in Uschinsky's and Fermi's solutions changing them to a pale green color in three to five days; faint growth occurs in Cohn's solution. Plain milk is cleared without coagulation, the cleared fluid becoming a pale turtle-green color; litmus milk becomes deeper blue. Gas is not produced from the ordinary sugars in Dunham's solution. Nitrates are not reduced, and some indol is formed.

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* Smith, Erw., Proc. Am. Asso. Adv. Sci., 46, 228–290, 1897.

† Brown, Nellie A., "A Bacterial Disease of Lettuce," Jour. Agr. Res., Vol. IV., No. 5, p. 475, 1915.

METHOD OF INFECTION.—Inoculation experiments indicate that infection may take place either through the stomata or through wounds produced by mechanical injury.

CONTROL.—No control measures have been reported.

BLIGHT OF MULBERRY

Pseudomonas mori—Boyer and Lambert (Smith)

HISTORY.—The disease was first studied in 1890 by Cuboni and Garbini in Italy, and later by Boyer and Lambert in France who named the causal organism *Bact. mori,* but did not describe it. In 1908, Erwin F. Smith* found a similar disease in some of the Southern States, and described the causal organism.

SYMPTOMS.—According to Erwin Smith, the blight attacks the leaves and young shoots of the mulberry, producing first water-soaked spots, which later become sunken and black; "foliage more or less distorted; shoots soon show sunken black stripes and dead terminal portions. Action of disease rather prompt." In very young shoots, wood, pith and bark are invaded by bacteria; in older shoots the germs are confined mostly to the xylem.

CAUSAL ORGANISM.—The organism is a rod with rounded ends, 3.6μ by 1.2μ, motile by 1 to 2 polar flagella, attached to one end. No spores observed; pseudozoögloea occur. Stains readily with carbol fuchsin; Gram-negative.

On agar, spreading, smooth, dull, translucent, shiny, white; medium not stained.

On potato, spreading, glistening, smooth, white to dirty white, shiny, medium grayed, slight action on starch. Gelatin stab, filiform, no liquefaction. Beef broth, pellicle, strong clouding. Milk, no coagulation, rendered alkaline, becomes clear by solution of fat and casein, litmus not reduced. No growth or scant in Cohn's solution. Uschinsky's solution, copious, pellicle, not viscid fluid, bluish-fluorescent color. No gas from dextrose, saccharose, etc. Aerobic. No indol or slight. Nitrates not reduced. Thermal death-point 51.5°; does not grow at 37°.

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* Smith, Erwin F.: Bacterial Blight of Mulberry, Science N S., Vol. XXXI, 803.

Blade Blight of Oats

Pseudomonas avenæ—Manns and *Bacillus avenæ*—Manns*

History and Distribution.—A specific bacterial disease of oats has been described by Manns in 1909. What appears to have been a similar trouble, extending from the Atlantic coast west to Indiana, and from the Great Lakes to the Gulf States, was observed as early as 1890 by Galloway and Southworth. Its appearance was noted for the first time in Colorado in 1915.

Symptoms.—In the early stages of the disease there is "a yellowing, beginning either as small round lesions on the blade, or as long, streak lesions extending throughout the blade or even the whole length of the culm and blades. In the advanced stages, the affected blades take on a mottled to almost red color, which has been called 'rust' and 'blight.'"

Cause of the Disease.—The disease is produced by the symbiotic growth of two bacteria whose activity is favored by rainy, humid, and cloudy weather. One of these organisms, *Ps. avenæ*, alone, is said to be capable of effecting the blight in a mild form, while the other, *B. avenæ*, is nonpathogenic; but a mixture of the two germs results in an aggravated attack.

Method of Infection.—Infection takes place through the stomata, the organisms being spattered on the leaves from the soil by rains. Grain insects are also responsible for spreading the disease.

Control.—It is believed that the control of the disease lies in the selection of resistant strains.

Stem Blight of Field and Garden Peas

Pseudomonas pisi—Sackett

History and Distribution.—The disease occurs in several of the Western States, particularly in the mountain valleys of the higher altitudes. It was first observed in Colorado in 1915, where it caused a loss of approximately one-third of the field peas in the San Luis Valley, while in other parts of the State where garden peas are grown for canning purposes, the crop was materially affected.

Symptoms.—The plants usually show the infection before they are 8 inches high, and many succumb before they reach that size. Both field and garden peas are affected alike, and the symptoms simulate the bacterial stem blight of alfalfa. The stems have a watery, olive-green appearance which soon becomes olive-brown, and in the last stages dark brown. The leaves and stipules appear watery at first, as if bruised, and later turn ocher yellow in color; this is often accompanied by wilting. In young plants, the discoloration of the stems is followed by a shrivelling, and ultimately the plants dry up and die; in the older ones, where the infection has taken place later, the same condition may result, but on the whole, the disease appears to be less serious, and in some cases the plants seem to outgrow the blight. Frequently when the first and earliest shoots are destroyed, the plant throws up new shoots from below ground, and a good late crop is obtained, in spite of the trouble.

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* Manns, "The Blade Blight of Oats, A Bacterial Disease," Bull. 210, Ohio Exp. Sta., 1909.

CAUSAL ORGANISMS.—*Pseudomonas pisi*, n. sp., as described by Sackett,* is a short rod with rounded ends, motile by means of a single polar flagellum; neither spores nor capsules observed; filaments formed commonly; stains readily with aqueous stains, and is Gram-negative.

It produces a flaky surface scum with heavy clouding in broth. On nutrient agar the growth is smooth, glistening, grayish white, and the medium is not discolored. Gelatin is liquefied rather rapidly. On potato, smooth, glistening, cream to orange-yellow; medium becomes grayish brown. No growth in Cohn's or Uschinsky's solutions. Heavy clouding with white surface pellicle in Fermi's solution; clouding with surface scum in Fraenkel's solution; slight, transient clouding in Naegli's solution. Plain milk is coagulated, and the coagulum is slowly peptonized, the supernatant liquid becoming yellowish green. Litmus milk becomes bluer, and the litmus is reduced, the liquid becoming greenish-gray. Neither indol nor hydrogen sulphide is produced. Ammonia is produced from asparagin and peptone. Nitrates are not reduced. No gas is formed from sugars, but acid is produced from dextrose, saccharose and galactose. Obligative aerobe. Optimum temperature 25° to 28°. Thermal death-point 50°. Habitat, soil.

Pathogenic for field pea and garden pea (*Pisum sativum* var. *arvense* and *Pisum sativum*).

METHOD OF INFECTION.—Experimental inoculations indicate that infections take place either through the stomata or through wounds produced by mechanical injuries.

CONTROL.—There seems to be a close relation between the prevalence of the disease and a late, cold spring. The low temperatures appear to make the plants more susceptible, and as a result the early plantings are the worst affected. As a control measure, planting from two to three weeks later is suggested.

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* Sackett, Walter G., "Stem Blight of Field and Garden Peas—A Bacterial Dieases". Bull. 218, Colorado Exp. Sta., April, 1916.

PEAR BLIGHT

Bacillus amylovorus—(Burrill) De Toni

HISTORY AND DISTRIBUTION.—As early as 1780, William Denning, a fruit grower, who lived on the Highlands of the Hudson River, observed pear blight in the trees of his neighborhood. It is very probable that blight existed many years before this in eastern North America on some of our native wild crabs, hawthorns, and wild plums, and with the introduction of cultivated varieties, it found a new field for attack. As the farming communities became more thickly populated, and the orchards more numerous, it has spread gradually westward over the

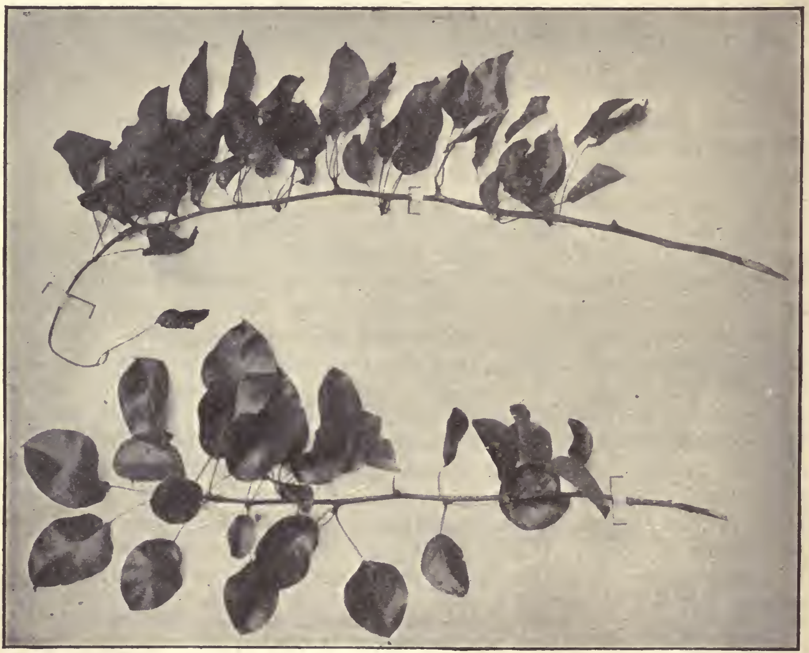

FIG. 196.—Two pear twigs. The upper one affected with Fire Blight, the lower one healthy. (*After Sackett, Mich. Agr. Exp. Sta.*)

Allegheny Mountains into the Mississippi Valley, across the Great Plains, and over the Rocky Mountains to the Pacific Coast. So generally is it distributed over the United States and Canada that a blight-free orchard is, indeed, a rare sight. The disease has progressed with such severity that, to-day, commercial pear growing in Colorado has been practically abandoned, and the industry in California is being threatened with destruction. So far as our present knowledge goes, the blight is of American origin and is confined to North America.

OCCURRENCE.—While the ravages of the disease are worst upon the pear, from which fact the disease derives its name, many varieties of the apple, quince, apricot and plum, together with the mountain ash, service berry, wild crabs and several species of hawthorn, have suffered severely from the same cause, and are capable of transmitting the disease from one to the other.

SYMPTOMS.—The disease is most easily recognized during the growing season, when it attacks the blossom clusters and the tips of the growing twigs. In this form it is known as *blossom* and *twig* blight. The leaves attached to these parts usually turn brown or black, either wholly or in part, the petioles blacken, and the young twigs show a blackened, shriveled bark, having much the appearance of green brush which has been burned only partially. It is from these symptoms that we get the name *Fire Blight*, so appropriately applied to pear blight. The blackened, withered leaves cling tenaciously to their blighted twigs long after the other leaves have fallen in the fall, and in this way afford the orchardist an easy way of recognizing the blighted areas.

Frequently the disease finds its way into the larger limbs and even the trunk of the tree, where it produces *body* blight. This form is characterized in the early stages by a cracking of the bark and the oozing of a thick, dirty white or brown, sticky liquid which collects here and there in drops over the injured surface. As the disease progresses, the splitting of the bark increases and the area involved becomes rough, giving rise to a canker. This is not to be confused with sun scald, in which the bark dries down and adheres firmly to the wood beneath, and which is due to an entirely different cause.

The immature fruit manifests the blight by turning black, shriveling and taking on a dried, mummified appearance. Accompanying these changes, drops of a thick, sticky exudate usually appear on the surface.

If a cross section is made of a diseased twig or limb, one invariably finds a blackened ring in the region of the cambium layer. This phenomenon, the significance of which will be explained later, serves as a reasonably reliable means of diagnosis.

Cause.—A microscopic examination of either the blackened cambium or a drop of the exudate shows swarms of motile rods, *B. amylovorus*, which Burrill of the University of Illinois, as early as 1878, credited with being the cause of pear blight. By inoculating healthy trees with this gummy material, he was able later to demonstrate his point experimentally, and with his work and that of a Dutch botanist, Wakker, we have the beginning of the study of bacterial diseases of plants.

Methods of Infection.—The more careful observers believe that insects, especially bees, plant lice and twig borers are responsible for the initial infection and subsequent spread of the disease. It has been found that the bacteria find protection from the adverse conditions of winter in the margins of the old cankers next to the sound bark, and also in some of the blighted shoots and twigs.* These hold-over bacteria become active with the increased flow of sap and the higher temperature of spring, and soon spread into the adjacent healthy bark. Here they multiply so rapidly that at about the time† the trees are in blossom, they begin to ooze from the cracks in the diseased bark as drops of a thick, sticky material, dirty white or brown in color. Insects are attracted to this ooze, apparently feed upon it, smear their feet, bodies and mouth parts, and then fly away to the opening blossoms. Here they feed upon the nectar and while so doing infect the flowers. The germs increase rapidly in this sweet liquid, and each bee that visits the flower subsequently carries away millions of germs to infect other blossoms. From the flowers, the bacteria find their way into the cambium and softer tissues of the bark, where the disease is confined almost entirely. After about ten days the progress of the germs can be noted by the blackening of the flower clusters, and the wilting and blackening of the leaves of the fruit spurs. Following the collapse of the fruit spurs, the disease may move down the twig an inch or more a day, causing it to appear watery, turn black and shrivel. The blackening may be 10 to 12 inches behind the advancing infection.

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* The writer examined a number of blighted pear twigs Apr. 14, 1911, collected from different orchards in Colorado and found *B. amylovorus* alive in 23.53 per cent. The germs occurred in the 2 cm. adjacent to the healthy part of the twigs.

† Whetzel, Bull. 272, Cornell Exp. Station, 1909.

This may continue until the whole limb becomes involved, but as a rule it is only the smaller twigs which are the worst affected. From this it will be seen that the external blackening cannot be relied upon, early in the season at least, as a guide to the exact location of the disease; however, as the season advances, the plant tissues harden, conditions for germ life become less favorable, and as a result, by the middle of summer, the active progress of the blight is checked by natural causes, and the blackening overtakes the advancing infection.

Blight which appears on the water sprouts of large limbs later can usually be accounted for by inoculation by plant lice and the pear twig borer.

CAUSAL ORGANISM.—According to Jones* *Bacillus amylovorus* possesses the following characteristics: Short motile bacillus, rounded ends, 1μ–1.8μ by 0.5μ–0.9μ; stains readily with the aqueous stains; Gram-negative. No spores observed.

Agar slant and potato, growth moderate, filiform, glistening, smooth, grayish white, semi-opaque, butyrous. Gelatin stab, growth rather slow, filiform, slight crateriform liquefaction after twenty days. Nutrient broth, moderate clouding, uniform; if left *undisturbed*, a delicate pellicle or ring may form which breaks up and sinks with the slightest jar; scant finely granular sediment after ten days. Litmus milk, light blue in four days, pinkish in six days, light blue again in twelve days, upper layer blue in eighteen days; soft gelatinous curd six to ten days, with whey on the surface. Cohn's solution, no growth. Uschinsky's solution, no growth. Nitrates not reduced. No indol. Thermal death-point 50°. Optimum temperature 23° to 25°. Slight acid production but no gas from dextrose, etc. Starch is not fermented.

CONTROL.—It is obvious that spraying is useless for a disease of this character, where the germs are located beneath the surface.

A systematic cutting out of the diseased limbs and twigs wherever and whenever they appear is the only practical method of controlling the blight. It is almost impossible to get all of the diseased material in the summer time when the heavy foliage hides it, but in the fall and winter the blighted branches can be recognized very readily by the tufts of dead leaves clinging to them. It is necessary in removing the dead wood to cut well below the discolored part, 10 to 15 inches, for the bacteria may be considerably in advance of the discolored area. Clean out all old cankers by cutting well into the healthy part and by removing the dried, diseased material. Disinfect the freshly cut surfaces of this wound as well as the exposed ends of twigs and limbs with

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* Jones, D. H., The Bacterial Blight of Apple, Pear and Quince Trees. Bull. 176, Ontario Agr. College.

1:1000 solution of mercuric chlorid. All diseased wood must be collected and burned.

STREAK DISEASE OF SWEET PEAS AND CLOVERS

Bacillus lathyri—Manns and Taubenhaus

HISTORY.—The first recorded observations of this disease were made by Diggs on sweet peas in Dublin, Ireland, in 1904. The trouble was known locally as "Streak" disease of the sweet pea, and various parasitic fungi were assigned as the cause. One investigator even ventured the assertion that the malady was of a physiological nature. In 1912, Taubenhaus isolated a bacillus from clovers and sweet peas collected in the vicinity of Newark, Delaware, and which bore lesions similar to those described for "Streak." Subsequent inoculations with pure cultures proved the disease to be of bacterial origin and identical with that observed in England and Ireland.

SYMPTOMS.—The disease makes its appearance during the season of heavy dew and is characterized by light reddish-brown to dark brown spots and streaks, almost purple when old, along the stems. They usually originate near the ground, which seems to indicate distribution by spattering rain and infection through the stomata. The disease is quickly distributed over the more mature stems, and ultimately the cambium and deeper structures are destroyed in continuous areas resulting in the premature death of the plant. Occasionally the petioles and leaves show the infection; the latter exhibit the water-soaked areas common to bacterial stomatal infections such as are met with in alfalfa blight and bacteriosis of beans.

CAUSAL ORGANISM.—Manns and Taubenhaus have described the organism which is responsible for the disease as a new species under the name *Bacillus lathyri.* It is a small rod, motile by means of 8–12 short, peritrichiate flagella; it grows luxuriantly upon all of the common nutrient media, especially if sugars are present, producing a yellow pigment; on glucose agar, colonies appear in twenty-four to thirty-six hours, showing a tendency to become stellate or auriculate.

PATHOGENESIS.—*Bacillus lathyri*, n. sp. has been isolated from specific lesions on the following hosts: Sweet pea, Lathyrus spp., red, alsike and mammoth clovers, soy beans, garden beans, cow peas and alfalfa.

METHOD OF INFECTION.—Infection appears to take place through the stomata, the organism being spattered on the plants from the soil during rains.

Control.—On small areas, heavy mulching of straw along either side of the row is suggested as a possible means of preventing the distribution of the disease.

Tomato Blight

Bacterium (?) *michiganense*—Erw. Smith*

The disease is distinct from the wilt, caused by *B. solanacearum*, in that there is not the sudden collapse of the whole plant, but rather a slow yellowing or wilting of the leaves, one at a time. The causal organism produces cavities in the pith and bark as well as in the vascular system.

Walnut Blight or Bacteriosis

Pseudomonas juglandis—Pierce

History and Distribution.—Attention was first called to this disease as it occurred in California by Pierce† in 1893 although it had been observed in Los Angeles County in about 1891. Outside of California, it is known to occur in Oregon, Texas and midway down the Pacific coast of Mexico. What appears to be a similar trouble has been reported from New Zealand and France.

Symptoms.‡—All of the new, tender, growing parts of the tree, such as young nuts and branches, petioles of leaves, midveins, fine lateral veins and adjoining parenchyma are subject to the attacks. On the branches, the disease always starts in the young succulent growth and manifests its presence by small, discolored areas which under favorable conditions may extend 2 to 3 inches along the green shoot. As the infection progresses, the central portion of the lesion turns black and is surrounded by a water-soaked margin. In the later stages, the whole diseased area becomes blackened and in many instances has a somewhat shrunken, dried-out, deformed, cracked appearance due to the drying out of the tissue. In severe cases the tissue is killed inwardly to the pith, while in the milder attacks only the bark and wood are diseased. As the wood hardens, the infection is checked, and the vitality of the tree is not affected to any extent, the crop suffering rather than the tree. The leaves sometimes exhibit a blackening or browning of the petioles and veins, while the intermediate tissue may develop brownish, circular or angular spots. The disease does not cause serious defoliation of the tree. The catkins are probably not affected. It is upon the young nuts that bacteriosis is especially destructive, and it would be of little economic importance did it not attack these. Many of the nuts may become infected and fall when they are ⅛ to ½ inch in diameter and continue to drop throughout the summer. A conservative estimate of the loss places it at 50 per cent in badly diseased groves. The most common point of infection is at the blossom end, although, it may start at any place on the nut. In

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* Smith, Erw., Science, N. S., Vol. XXXI, 803, p. 794, 1910.

† Pierce, N. B., Bot. Gaz., 31; 272–273, 1901.

‡ Smith, C. O., "Walnut Blight," Bull. 231, Calif. Exp. Sta., 320, 1912.

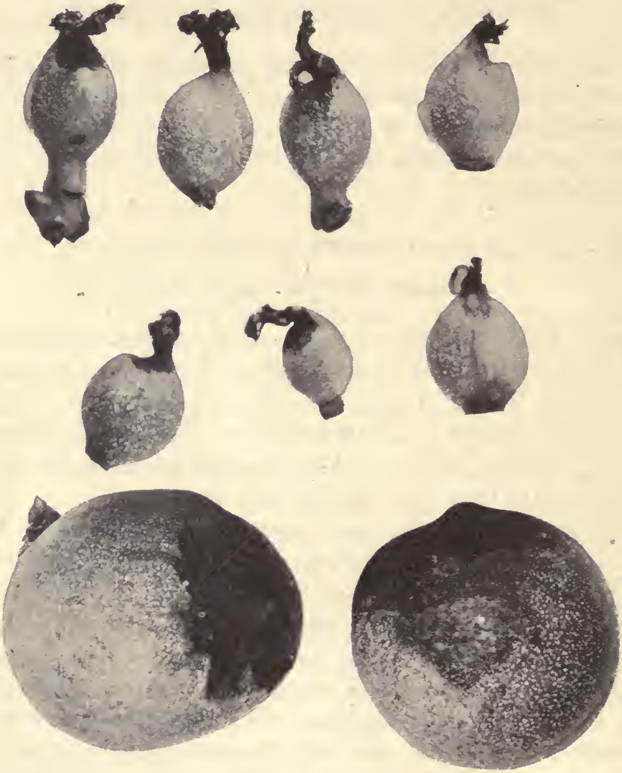

FIG. 197.—Walnuts affected by Bacteriosis, mostly stigma or blossom-end infection. (*After C. O. Smith, Calif. Bull.* 231.)

the early stage, the lesions appear as small, circular, raised, discolored, water-soaked areas; later, these spots increase in size and turn black. Under favorable conditions, the disease may extend through the hull and shell-forming tissues into the kernel which at length becomes blackened and finally destroyed.

CAUSAL ORGANISM.—According to Smith, C. O., *Pseudomonas juglandis*, Pierce, is a rod with rounded ends; single or in pairs, rarely in chains; measures 1.5μ to $3.01\mu \times 0.3\mu$ to 0.51μ; stains readily with the ordinary aniline dyes; Gram-positive; spores and capsules not observed; motile by means of a single polar flagellum; agar colonies nucleated, circular, moist, shining, pale yellow with regular margins; startiform liquefaction in gelatin; potato, abundant, moist, shining, slimy, raised, white changing to yellow; uniform turbidity and ring in bouillon, slight flocculent precipitate; indol produced; nitrates not reduced; enzymes, diastasic, cytohydrolytic, rennet, proteolytic; milk coagulated, curd digested; litmus milk wine colored; viability, nine and one-half months on potato; methylene-blue milk reduced.

METHOD OF INFECTION.—It has been shown that the causal organisms live over winter in the old lesions of the wood and bark and that in the spring they exude to the surface and are carried to the new growth, to which they gain entrance through the stomata. The disease is most severe during seasons when the fogs and rainfall are heaviest, and in those localities where rain and fogs are abundant. "During one of these fogs the trees become saturated, water dripping from one portion of the tree to another which could easily carry the disease organisms to healthy tissue." Distribution by this means is thought to be one of the most important, if not the most important, methods of spreading the trouble. Insects probably play some part in the dissemination of blight.

PATHOGENESIS.—Pathogenic for *Juglans regia* (English) under natural conditions; pure culture inoculations give positive lesions on *Juglans nigra* (eastern black), *Juglans hindsii* (northern Cal. black), *Juglans californica* (southern Cal. black), *Juglans cinerea* (butternut).

CONTROL.—Systematic spraying experiments with Bordeaux mixture, lime-sulphur, and a sulphur spray have demonstrated that spraying is impracticable and has little value as a means of control. Applications of lime to the soil have resulted in no benefit. It has been observed that individual trees exhibit great differences in their natural resistance to the blight, and at the present time the selection and propagation of varieties which are more or less immune promises the most practical solution to the problem.

CHAPTER II

GALLS AND TUMORS

Crown Gall

Pseudomonas tumefaciens.—Erw. Smith and Townsend

Crown gall is one of the most recent plant diseases to be traced to bacterial origin. Its occurrence is so common in nursery stock that in a certain Western State, 75 per cent of the young trees and shrubs condemned by nursery inspectors are condemned for crown-gall, and Toumey places the annual loss to orchardists at $500,000 to $1,000,000.

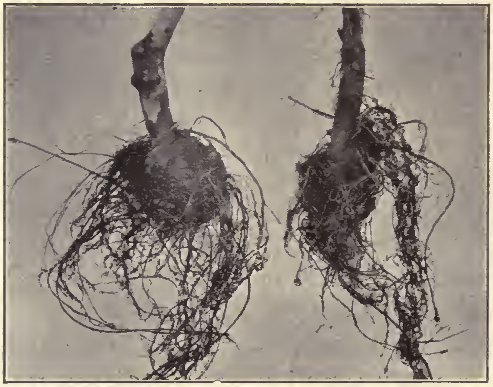

Fig. 198.—Crown gall with hairy root on nursery stock. Northern Spy apple. (*After Paddock.*)

History.—Smith and Townsend* working with the gall of the Paris daisy observed bacteria in these outgrowths in 1904, but it was not until 1906 that they succeeded in isolating the causal organism and in securing satisfactory re-inoculations. Subsequent studies* have shown that this same microörganism is responsible for the pathological condition that we recognize as crown gall in its various forms on the different hosts. One of the remarkable things about this disease is the large number of families which are subject to the infection.

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* Smith, Erw. F., and Townsend, C. O., "A Plant Tumor of Bacterial Origin," Science, N. S. Vol. XXV, No. 643, p. 671–673, 1907; "The Etiology of Plant Tumors," Science, N. S. Vol. XXX, No. 763, p. 233, 1909.

Townsend, C. O., "A Bacterial Gall of the Daisy and Its Relation to Gall Formations on Other Plants," Science, N. S. Vol. XXIX, p. 273 (Abstract), 1909.

Pathogenesis.—A partial list of the plants upon which crown gall occurs naturally or upon which it has been produced by laboratory inoculation includes the daisy, tomato, tobacco, potato, carnation, peach, rose, cabbage, grape, hop, sugar-beet, turnip, red beet, carrot, radish, chrysanthemum, oleander, marigold, pyrethrum, almond, clover, white poplar, Persian walnut, Pterocarya, gray poplar, cotton, alfalfa, raspberry, geranium, apple, willow, quince.

Symptoms.—The swellings or galls, small at first, usually appear just below the ground line (crown), at or near the juncture of the stock and scion. These may be either hard or soft galls; the former are smooth, soft, spongy, white to flesh-colored outgrowths which may reach a very appreciable size during one season and then be entirely decomposed and disappear by the following spring; the latter increase in size more slowly, persist year after year, harden and become rough and warty on the surface with age. Both are crown galls and both are produced by bacteria. According to Smith,† "Whether a crown gall shall develop as a hard gall or a soft gall would seem to depend chiefly if not altogether on which meristem cells receive the initial impulse. If the cells first infected are principally the mother cells of medullary rays, we may assume that the gall will be a 'soft gall,' and readily inclined to decay. If, on the contrary, the needle or other carrier of infection wounds principally those meristem cells which give rise to tracheids and wood fibers, the gall will be a 'hard gall,' of slow growth and long duration." The structure of the galls is unlike that of club-root of cabbage in that the latter is an hypertrophy while the former is an hyperplasia. Frequently this disease assumes a form known as "hairy root" characterized by the presence of bunches or tufts of closely matted rootlets with enlargements at their bases. As the galls‡ enlarge, the function of the adjacent conducting tissue is interfered with, and the circulation is impaired, as is shown by the poor growth and dwarfed appearance of the trees.

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* Smith, Erw. F., Brown, Nellie A., Townsend, C. O., "Crown-gall of Plants: Its Cause and Remedy," Bull. 213, Bur. Plant Ind., U. S. Dept. Agr., 1911.

† Smith, C. O., "Further Proof of the Cause and Infectiousness of Crown Gall," Bull. 235, Calif. Exp. Sta., 1912.

‡ Very hairy roots often accompany these.

The development of this disease is looked upon by Smith* and his associates as paralleling closely what takes place in cancer in man and animals. The primary tumors have been observed to send out "roots" or tumor-strands for some distance into the normal tissue, and from these tumor-strands, secondary tumors may arise which tend to take on the structure of the primary tumor, *e.g.*, "if the latter is in the stem and the former in a leaf, the secondary tumor shows a stem structure." "There are no metastases in crown gall * * * for whether a cancer shall be propagated by floating islands of tissue, or only by tumor-strands, appears to be a secondary matter depending upon the character of the host tissues rather than the nature of the disease." The salient point is the internal stimulus to cell division which arises from the presence of the microörganisms within certain cells.

METHOD OF INFECTION.—Little is known about the natural channels of infection, but inoculation through wounds induced by poor grafting, careless cultivation, and by borers, nematodes, etc., is undoubtedly responsible for many crown galls.

CAUSAL ORGANISM.—*Pseudomonas tumefaciens* is a short rod with rounded ends, motile by 1–3 polar flagella; measures 1.2 to 2.5μ by 0.5 to 0.8μ; neither spores nor capsules demonstrated; pseudozoöglœæ occur; involution forms present; stains with the usual anilin stains; Gram-negative; on agar, slow, four to six days at 25°; filiform, raised, white, glistening, somewhat slimy; potato, growth rapid, white, smooth, wet-glistening; gelatin stab, filiform, no liquefaction; moderate, flat, filiform, white, smooth, glistening, no liquefaction; blood serum, moderate; broth, ring or pellicle, clouding absent or inconspicuous; milk, coagulation delayed, curd not peptonized, litmus gradually blued then reduced; silicate jelly, slow white growth; Cohn's solution, scanty or absent; Uschinsky's solution, scanty, not viscid; NaCl bouillon, 4 per cent inhibits, 3 per cent retards; bouillon over chloroform, growth unrestrained; no gas from sugars; ammonia is produced; nitrates not reduced; indol production small; thermal death-point 51°; optimum reaction between +14 and +24 Fuller's scale; opt. temp. 25° to 28°, max. 37°, min. positive at 0°; killed readily by drying; moderately sensitive to sunlight; invertase and rennet thought to be produced.

CONTROL.—Thorough inspection of nursery stock and care in the cultivation of orchards not to wound the crowns are important factors.

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* Smith, Erw. F., Brown, Nellie A., McCulloch, Lucia, "The Structure and Development of Crown Gall; A Plant Cancer. Bull. 255, Bur. Plant Ind. U. S. Dept. Agric., 1912.

Plant on uninfected land and avoid heeling in healthy stock into soil that has previously borne diseased plants.

Removing the galls results in no practical benefit.

OLIVE KNOT

Bacterium savastanoi—Smith*†

The olive knot has been known for many years, and is even described by the early Roman writers; its bacterial nature, however, has been recognized only since 1886. It is most prevalent in those countries which border on the Mediterranean Sea, but it also occurs in the olive growing sections of California.

So far as is known, the causal organism enters the twigs and leaves of the olive through wounds, and there produces roughened, wart-like swellings. The growth of the knots usually begins in the spring, and later in the season, if the trees are badly diseased, they show scant foliage, limited growth, and occasionally dead branches, especially where the galls have entirely encircled the twigs.

"FINGERS AND TOES" OR "STUMP ROOT" OF CABBAGES‡

Plasmodiophora brassicæ—Woronin (1877)

This organism which is classified as a rhizopod by many is the cause of a common disease of the roots of cabbages and of other cruciferous plants. The disease is sometimes called "fingers and toes." It may cause much damage in market gardens. In it the roots are greatly hypertrophied. They are distorted and lumpy, like fingers bent and swollen with rheumatism. The disease may be controlled to some extent through the destruction, by burning, of all infected material as soon as the disease is recognized.

It is usually considered well to rely on the rotation of crops or, in case the soil has become generally infested, to plant crops of another type for several years in order to prevent losses from this infection. The plants attacked are recognized by their stunted appearance and by the tendency of the leaves to wilt or turn yellow. A microscopial examination of the roots will reveal the distinctive protozoa which cause the disease.

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* Smith, Erw., Bull. 131, Part IV Bur of Plant Industry, U. S. Dept. of Agriculture, 1908

† Savastano, L. Les maladies de l'olivier et la tuberculose en particulier. Comp. Rend. 103 1144, 1116. Il bacillo della tuberculosi dell'olivo, nota suppletiva. Rend. Lincei 5:92–94 1889.

‡ Prepared by J. L. Todd.

The spores are liberated with the disintegration of the diseased roots and become disseminated in the soil during cultivation. Under appropriate conditions the spore is ruptured and a small flagellated, amœboid organism emerges. It is in this form that the parasites penetrate the roots of the young plants in which they complete their development. The youngest forms seen within the vegetable cells possess two nuclei each with a central mass of chromatin or karyosome. Several organisms frequently invade a single cell. As they grow, there is a multiplication of nuclei and the associated organisms tend to fuse together to form plasmodia. Subsequently there occurs a series of changes. Some of these changes are readily distinguished; but others are more difficult to follow. The nuclei first lose the greater part

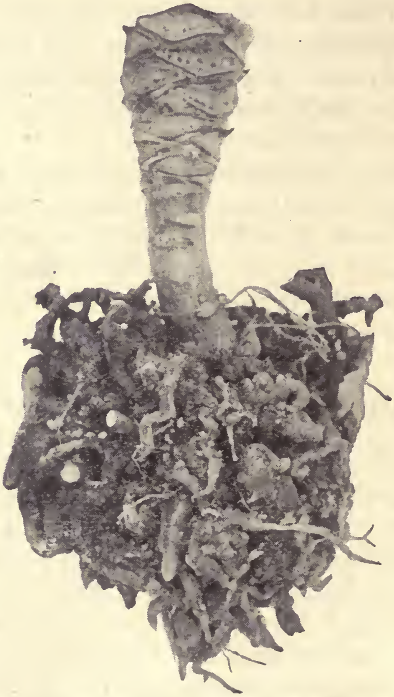

FIG. 199.—Roots of Cabbage plant showing characteristic hypertrophy due to *Plasmodiophora brassicæ*. (*Woronin.*)

of their chromatin and appear pale and indistinct, while attraction spheres appear at opposite poles. The nuclei then divide twice by karyokinesis and a small amount of cytoplasm is separated off, constituting a gamete. The gametes now unite in pairs and each pair becomes encysted to form a spore.

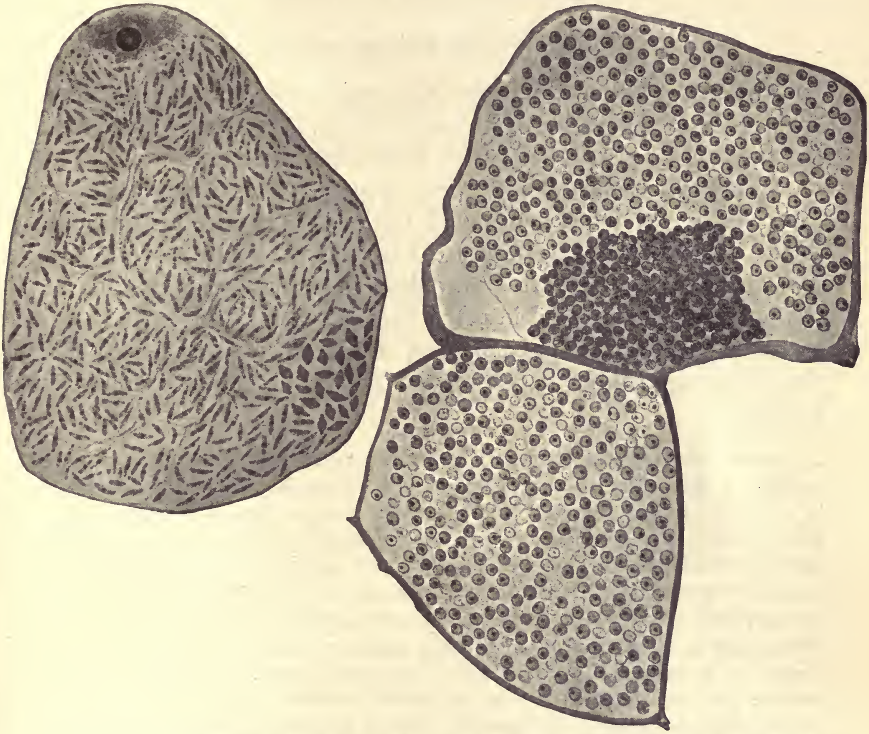

FIG. 200.—*Plasmodiophora brassicæ*. *A*, A plant cell filled with parasites the nuclei of which are undergoing mitotic division (at the top is the nucleus of the plant cell). *B*, Two plant cells with developed and partly developed spores. (*After Prowazek, from Doflein.*)

Whether during the multiplication of these organisms in the plant, they are able to migrate to other cells and thus spread the infection has been questioned. A number of investigators believe that the number of infested cells is only increased by the division of the infected plant cells which not only are greatly enlarged but also show evidence of proliferation in the presence of dividing cells. The hypertrophy of the plant cell is associated with hypertrophy of its nucleus and it is evident that the growth and increase of the parasite is favored by the reaction which its presence excites.

TUBERCULOSIS OF SUGAR-BEET

Pseudomonas beticola—Smith

HISTORY.—This new disease of the sugar-beet, resembling somewhat crown gall on the surface, but distinct from it, was first observed in the autumn of 1910 on beets from Colorado and Kansas.

SYMPTOMS.—Affected beets bear numerous wart-like outgrowths or tubercles on the upper portion of the root. On section these show small, water-soaked, brownish areas with more or less necrotic tissue in their interiors; such areas may develop small central cavities, and the softening may extend into the ungalled part of the beet; the diseased parts appear mucilaginous and stringy when touched, and under the microscope this broken-down tissue is found to be swarming with bacteria.

CAUSAL ORGANISM.—According to Smith* *Pseudomonas beticola*, n. sp., is a motile rod with rounded ends, single or in pairs, chains or clumps; measures 0.6 to 0.8 by 1.5 to 2.0μ; flagella polar; no spores observed; capsule present; liquefies gelatin, but not blood serum; grows in beef bouillon containing 9 per cent NaCl; uniform clouding and copious pellicle which falls easily in bouillon; thermal death-point 51°; grows at 37° but best at 20°; grows slowly at 1°; produces a yellow rim and pellicle in plain milk which is slowly coagulated; whey separates slowly; litmus milk is blued and later reduced; grows readily in Uschinsky's solution, viscid; no growth in Cohn's solution; moderate growth on potato; does not produce gas from dextrose, lactose, saccharose, maltose, mannite or glycerin; agar colonies, circular, smooth or wrinkled; indol is produced; grows in bouillon over chloroform; resists drying; stains by Gram; is yellow or becomes yellow on all ordinary media.

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* Smith, Erwin F., "Crown Gall of Plants: Its Cause and Remedy." Bull. 213, Bur. Plant Ind., U. S. Dept., Agr., p. 194, 1911.

CHAPTER III

LEAF SPOTS

CITRUS CANKER

Pseudomonas citri—Hasse

The disease was probably introduced into the United States on nursery stock from Japan, and since 1912, has occurred in Florida, Alabama, Mississippi, Louisiana, and Texas.

SYMPTOMS.—According to Stevens,* citrus canker attacks all varieties of citrus trees of any commercial value in Florida, but it is most severe on the grape fruit. Under field conditions a characteristic spotting of the fruit, foliage and twigs is produced which appears as small light-brown spots, 1.5 mm.–6 mm. (1/16 to 1/4 inch) in diameter. These spots may occur singly or several may coalesce to form an irregular area; they are raised above the adjoining tissue and are made up of a spongy mass of dead cells, covered by a thin white or grayish membrane, which ultimately ruptures forming a ragged margin around the spot. The fruit is especially susceptible to the infection, and drops soon after it is attacked. The disease is spread rapidly from one part of the tree to another by insects, rains and heavy dews, so that when once infected, a tree frequently becomes worthless in two or three months.

CAUSAL ORGANISM.—Miss Clara H. Hasse† has described the causal organism, *Ps. citri*, as a short rod with rounded ends, motile by a single polar flagellum.

On nutrient agar, the growth is filiform, shining, dull yellow in color; on potato, bright yellow, shining, viscid. In nutrient broth, a yellow ring is formed at the surface in old cultures. Litmus milk becomes deeper blue, and the casein is precipitated. Gelatin is liquefied. Indol is not produced. No gas is formed from sugars in Dunham's solution. Growth is slight in Uschinsky's solution, and nitrates are not reduced in starch nitrate solution. The organism grows best under aerobic conditions.

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* Stevens, H. E., "Citrus Canker, I, II, III," Bulls. 122, 124, 128, Fla. Exp. Sta., 1914, 1915

† Hasse, Clara H., "*Pseudomonas citri*, the Cause of Citrus Canker," Jour. Agr. Res., Vol. IV, No. 1, p. 97, 1915.

METHOD OF INFECTION.—Experimental evidence goes to show that infection takes place through stomata as well as through wounds produced by insects, or by other mechanical injuries.

PATHOGENESIS.—According to Berger, the following citrus varieties are subject to citrus canker: Pomelo, citrus trifoliata, wild lime, Navel, sweet seedlings, Satsuma, tangerine, King orange and lemon.

CONTROL.—Removal of the affected parts of the tree by pruning has proven a complete failure as a control measure, and the only practical means of handling the disease appears to be the prompt and complete destruction, by burning, of all stock that shows the slightest trace of infection.

ANGULAR LEAF-SPOT OF CUCUMBERS

Pseudomonas lachrymans—Erw. Smith and Bryan

HISTORY AND DISTRIBUTION.—The angular leaf-spot of cucumbers is a widespread disease occurring in many of the Eastern and Middle Western States. It has been recognized in the field for more than twenty years, but it was not until 1914 that the causal organism was isolated.

SYMPTOMS.—The disease is characterized by the "numerous, often confluent, angular, dry, brown spots which tear or drop out when dry, giving to the leaves a ragged appearance. In the early stages a bacterial exudate collects in drops on the lower surface during the night and dries whitish,"* and because of these tear-like drops of exudate the specific name *lachrymans* has been suggested for the causal organism. The young stems and petioles may become soft-rotted and crack open, but there is little evidence that the fruit itself suffers from the disease, other than indirectly from lack of nourishment resulting from the destruction of the active leaf surface.

CAUSAL ORGANISM.—*Pseudomonas lachrymans* is a short rod with rounded ends, motile by means of 1–5 polar flagella. No spores have been observed; capsules are formed on agar and in milk. It is Gram-negative and is not acid fast.

On agar, the growth is smooth, shining, transparent, white; agar colonies, two to four days old, exhibit opaque white centers which spread in radiating lines into the thin margin. Gelatin is liquefied slowly, and as the liquefaction progresses the upper part becomes stratiform, the lower part bluntly funnel-shaped. In nutrient broth moderate clouding occurs, and a membranous pellicle is formed which breaks readily on shaking. On potato, the growth is slimy, shining, creamy-white. Plain milk clears slowly without coagulation, becoming translucent and tawny-olive with age. Lavender-colored litmus milk is completely blued in three days, and a creamy-white pellicle is formed at the surface; clearing is complete in twenty days, and later the blue color bleaches out leaving the fluid a translucent brown.

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* Smith, Erw. F. and Bryan, Mary Katherine: "Angular Leaf-spot of Cucumbers," Jour. Agr. Res., Vol. V, No. 11, pp. 465, 475, 1915.

The organism grows in Uschinsky's, Fermi's, and Cohn's solutions producing a green coloration in the first two.

No gas is formed from the ordinary sugars; acid is produced from saccharose and dextrose. Nitrates are not reduced. Hydrogen sulphid is not formed. A small amount of indol is produced in 2 per cent peptone water and peptonized Uschinsky's solution. Methylene blue in milk is rapidly reduced. The organism is an obligate aerobe.

Optimum temperature is 25° to 27°.; no growth at 36°.

METHOD OF INFECTION.—The causal organism enters the leaves through the stomata, no wounds being necessary.

CONTROL.—Laboratory experiments upon the germicidal action of copper sulphate on *Ps. lachrymans* suggest that Bordeaux mixture, properly applied, may be a remedy for the disease.

SPOT OF THE LARKSPUR

Bacillus delphini—Erw. Smith

So far as is known, this disease occurs only on the larkspurs of Massachusetts. Infection takes place through the stomata, resulting in numerous black spots on the leaves and stems.

CAUSAL ORGANISM.—Smith* describes the organism as a motile, gray-white, non-liquefying, nitrate reducing bacillus. Agar colony has characteristic wrinkled structure. Grows in Uschinsky's solution. No growth at 37°; thermal death-point 48° to 49.1°.

BACTERIAL SPOT OF PLUM AND PEACH

Pseudomonas pruni—Erw. Smith

The first occurrence of the bacterial spot was reported on the Japanese plum in Michigan.† Later, what appeared to be the same disease was observed on the peach in Georgia‡ and Connecticut, and more recently it has been found throughout the South and Middle West.

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* Smith, Erw. F., Science, N. S., Vol. XIX, No. 480, p. 418, 1904.

† Smith, Erw., Science, N. S., Vol. XVIII, 429, p. 456, 1903.

‡ Rorer, J. B., Science, N. S., Vol. XXIX, 753, p. 914, 1909.

SYMPTOMS.—On the plum, the leaves and green fruit exhibit numerous small, water-soaked spots; later the diseased tissue of the leaves falls out, giving a shot-hole appearance, and the plums show black, sunken areas and deep cracks. The spots may reach a diameter of one-fourth to one-half inch.

On the peach leaves, angular, purplish-brown spots one-eighth to one-fourth inch in diameter are formed, which drop out giving the shot-hole effect. The organism also attacks the young twigs and fruit. It destroys the bark of the former, producing black, sunken areas, while on the latter it causes small purplish spots over which the skin cracks.

In both the plum and the peach, infection is believed to take place through the stomata. It is primarily a disease of the parenchyma, but the vascular system is invaded ultimately.

CAUSAL ORGANISM.—*Ps. pruni* Smith, is a small rod, motile by one to several polar flagella. It grows readily upon the ordinary culture media. On agar, it resembles *Ps. campestris*, producing a distinctly yellow pigment, but is distinguished by its feeble growth on potato and by its growth in Uschinsky's solution, which is converted into a viscid material like egg albumin. Gelatin liquefied slowly. Casein of milk precipitated slowly and redissolved; litmus reduced but color restored later. No gas produced. Thermal death-point 51°.

DISEASE OF SUGAR-BEET AND NASTURTIUM LEAVES

Pseudomonas aptatum—Brown and Jamieson

HISTORY.—The bacterial leaf spot of sugar-beet and nasturtium leaves was first observed in the summer and spring of 1908 on nasturtium leaves growing near Richmond, Va., and on sugar-beet leaves obtained from Garland, Utah; more recently the trouble has been noted in California and Oregon on the sugar-beet.

SYMPTOMS.—Affected nasturtium leaves exhibit water-soaked and brownish spots from 2 to 5 mm. in diameter. The sugar-beet leaves disclose "dark-brown, often black, irregular spots and streaks from 3 mm. to 15 mm. in diameter. They occur on the petiole, midrib, and larger veins." Occasionally the discoloration extends along the veins, and the tissue on either side is brown and dry; sometimes cork-like protuberances occur at the central point of the spots. In badly diseased petioles the tissue softens as though affected with a soft rot, but where the infection is mild there is no indication of this condition.

Microscopic examination of the diseased spots and adjacent area shows the tissue to be filled with a large number of active bacteria. Sections cut from the central portions of the diseased areas show the cell walls to be ruptured or collapsed, while the cells bordering the ruptured places show that the bacteria are in the cells. The disease is reproduced readily with typical symptoms by means of needle prick inoculations with pure cultures. So far as has been observed, the causal organism does not attack the beet root, but is confined strictly to the beet leaf.

Causal Organism.—According to Brown and Jamieson,* *Pseudomonas aptatum*, n. sp., is a short, motile rod with rounded ends; flagella, bi-polar; involution forms rare; no spores or capsules observed; pseudozoögloeæ occur; aerobic; smooth whitish colonies on agar plate with fish scale-like markings; clouds beef bouillon in eighteen to twenty-four hours; produces alkaline reaction in litmus milk, with a gradual separation of whey from curd; liquefies gelatin; produces ammonia; no reduction of nitrates; fluorescence greenish; no diastasic action on potato starch; grows in Uschinsky's and Fermi's solutions; indol produced after ten days; optimum temperature 27° to 28°; maximum 34° to 35°; minimum 1°; thermal death-point 47.5° to 48°; vitality four to ten months in beef agar, ten to twelve months in beef bouillon, depending on temperature; growth good on litmus-lactose agar; growth much retarded on gentian violet agar; stains readily with basic anilin dyes; not acid fast; not stained by Gram; tolerates acids; oxalic 0.1 per cent; tartaric 0.2 per cent; hydrochloric 0.1 per cent; tolerates sodium hydroxide in beef bouillon, −18 Fuller's scale; no growth in Cohn's solution; killed readily by drying; not very sensitive to sunlight; retains its virulence two to three years.

Pathogenesis.—Pathogenic to nasturtium and sugar-beet leaves; spots have been produced by artificial inoculations on leaves of pepper, lettuce, egg plant, and upon the leaves and pods of the bean plant.

Method of Infection.—It is believed that infection takes place only in bruised or wounded tissue, due to insects or to mechanical injury.

Control.—No practical methods of control have been undertaken.

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* Brown, Nellie A., Jamieson, Clara O., "A Bacterium Causing a Disease of Sugar-beet and Nasturtium Leaves," Jour. Agr. Res., Vol. I, No. 3, p. 189, 1913.

CHAPTER IV

ROTS

Black Rot of Cabbage

Pseudomonas campestris—Pammel (Erw. Smith)

This disease is widely distributed in the United States and Europe and has become so serious on many truck farms that gardeners dread its appearance as much as orchardists do pear blight. It is not confined to cabbage, but it attacks other cruciferous plants such as cauliflower kohlrabi, kale, rape, turnips, mangels, rutabagas and mustards.

Symptoms.—The first symptom is the withered, yellow margin of the leaf, giving the impression of a "burned edge." The progress of the disease is inward and downward through the vascular system, as is indicated by the brown or black color of the veins and midrib. The tissue of the vascular bundles is destroyed and the cell walls of the adjacent tissue are dissolved, presumably by a cytolytic enzyme.* In this way practically all of the tissues are softened, disorganized, and a general infection of the whole plant may follow. Diseased leaves fall prematurely, leaving a long naked stalk with a tuft of leaves at the top. The dwarfed, one-sided growth of the heads, and in some cases the failure to produce heads is characteristic.

Method of Infection.—Water pore† infection along the margin of the leaf is believed to be the most common method of entrance, although root inoculation at the time of transplanting undoubtedly takes place also. It has been shown, further, that the germ is introduced on the seed.‡

Causal Organisms.§—*Pseudomonas campestris* Pammel, is a short rod with rounded ends, relatively shorter in the host tissue than on culture media, 0.7μ to

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* Smith, E. F.: Bull. 25, Bur. Plant Industry, U. S. Dept. Agr., 1903.

† Russell, E. J.: Bull. 65, Wisconsin Exp. Station, 1898. Smith, E. F.: Farmers' Bull. 63, U. S. Dept. Agriculture, 1898.

‡ Harding, H. A.: Bull. 251, N. Y. Experiment Station, 1904.

§ For a means of distinguishing *Ps. campestris*, *Ps. phaseoli*, *Ps. hyacinthi* and *Ps. stewarti*, the student is referred to Bull. 28, p. 149, Div. Veg. Phys. and Path., U. S. Dept. Agr., 1901.

3.0μ by 0.4μ to 0.5μ; motile when young by one polar flagellum; no capsule demonstrated and no spores observed; zoöglœæ in liquid cultures. Stains readily with aqueous stains. Gram-negative.

It grows readily in the ordinary culture media. Upon potato, growth is characteristic; at first light yellow, and in old cultures a golden brown, abundant, moist, shining, slimy. Gelatin liquefied slowly. Litmus milk becomes slightly alkaline, casein separated and gradually redissolved. On nutrient agar, translucent, yellow slime. No gas from dextrose, lactose, etc. Uschinsky's solution, growth retarded and feeble. Aerobic. Indol produced. Nitrates not reduced. Diastase produced. Optimum temperature, 25° to 30°; thermal death-point, 51.5°.

CONTROL.—The removal of diseased leaves in the early stages has been practiced by some growers with success, but care must be taken not to remove so many that growth will be checked. Manure containing diseased cabbage refuse must not be used. Seed disinfection with 1 : 1,000 mercuric chloride, fifteen minutes, or formalin 1 : 200, twenty minutes, is recommended. Rotation of crops, and planting on new land should be practised whenever possible. If practicable, the seed bed should be made in sterilized soil, so that the plants will be healthy when set in the field.

WAKKER'S HYACINTH DISEASE

Pseudomonas hyacinthi—Wakker

HISTORY.—One of the earliest landmarks in the study of bacterial diseases of plants is the excellent contribution of Dr. J. H. Wakker,* a Dutch botanist, who between 1883 and 1888 published five papers on a disease of the hyacinth, caused by *Ps. hyacinthi*. Erwin F. Smith† has carried the investigation farther and has described the causal organism more fully. The disease was first observed in the Netherlands where it frequently causes serious losses in the hyacinth gardens. It is not known to occur in any other part of the world.

SYMPTOMS.—The disease is characterized by a yellow striping of the green leaves and the bright yellow slime produced in the vascular bundles of the bulb. The infection in the leaf spreads slowly to the bulb by the multiplication of bacteria in the vascular system, filling the vessels, especially those of the bulb, with a bright yellow bacterial slime. In time, the walls of the vessels are destroyed and large cavities are formed in the fibro-vascular bundles. The disease does not spread rapidly from bundle to bundle in the bulb, but is confined for a long time to the vessels first involved, a year or more being required for the destruction of the host plant. This is due, largely, to the resistance offered by the cells of the parenchyma to bacterial invasion.

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* Wakker, J. H.: Bot. Centralbl., 1883, 14, p. 315; Archives neerlandaises des sci. ex. et naturelles, Tome XXIII, pp. 18–20.

† Smith, Erwin F., "Wakker's Hyacinth Germ," Bull. No. 26, U. S. Dept. Agr., Div. Veg. Phys. and Path., 1901.

METHOD OF INFECTION.—The causal organism enters through wounds in the leaves and through the blossoms, and when the disease is once established, it is probably spread by insects which visit the blossoms or eat the leaves. Daughter bulbs contract the infection from mother bulbs. Wakker believed the disease to be transmitted often by knives used around sick plants.

CAUSAL ORGANISM.—*Pseudomonas hyacinthi* Wakker, according to Erwin F. Smith, is a medium-sized rod with rounded ends, 1.0μ to 2.0μ by 0.5μ to 0.7μ, motile by one polar flagellum; non-spore forming.

It grows well upon the ordinary culture media, on most of which, as well as in the host plant, it produces a bright, chrome-yellow pigment. Gelatin and blood serum are liquefied slowly (six to seven days). Milk is rendered alkaline, and the casein is slowly precipitated. On nutrient agar, growth is copious, yellow, smooth, wet-shining, translucent, spreading. On 20 per cent cane agar, the zoöglœa formed gives the growth a papillose, verrucose appearance. Acid but no gas is formed in dextrose and saccharose broth; indol produced slowly. Nitrates not reduced. Feeble growth in Uschinsky's solution. Does not grow at 37°; optimum temperature 28° to 30°; thermal death-point 47.5°.

The hyacinth is the only known host plant.

CONTROL.—Diseased bulbs should be removed from the fields and destroyed; land on which the disease is present should be used for other crops; the use of infected tools without thorough disinfection should be avoided. The selection and breeding of disease resistant varieties, as advised by Wakker, suggests the most practical way of controlling the trouble.

BLACK LEG OR BASAL STEM ROT OF POTATO

Bacillus phytophthorus—Appel*

The disease is prevalent in the United States and Europe. It appears to originate in the seed tubers from which it extends upward into the base of the stem causing it to turn black and rot. The vines grow spindling, turn yellow and die prematurely. The diseased tubers may rot in the soil or later when in storage cause a soft rot of the crop.

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* Appel, Otto, "Untersuchungen u. d. Schwarzbeinigkeit." Arb. Bio. K. G. Amt., Berlin, 1903.

CAUSAL ORGANISM.—Erwin Smith describes the causal organism as a non-spore forming bacillus, motile by means of peritrichate flagella. It stains with the ordinary stains, but is Gram-negative. The growth is grayish-white on agar and on gelatin plates large, round, white colonies develop promptly. Gelatin is liquefied with funnel-shaped liquefaction. On cooked potato, white to yellowish growth. Raw potato, white growth and black stain. There is a slow acid coagulation of milk with precipitation of casein and reduction of litmus. Thick pellicle and heavy precipitate in potato juice. No growth in Cohn's solution. Moderate production of hydrogen sulphide. Nitrates reduced. No indol. Acid from dextrose, saccharose, lactose, maltose and galactose. Some gas from inosite, lactose and mannite. Facultative anaerobe. Optimum temperature, 28° to 30°. Thermal death-point, 47°.

Closely related organisms are *B. solanisaprus* Harrison, and *B. atrosepticus* van Hall.

CONTROL.—In view of the fact that the germs are introduced with the seed potatoes, thorough disinfection of the seed with formalin is recommended.

BUD-ROT OF THE COCOANUT

Bacillus coli (Escherich) Migula

HISTORY AND DISTRIBUTION.—The bud-rot of the cocoanut has been known for more than thirty years in Cuba and is to be found distributed more or less generally throughout tropical America and the eastern tropics.

SYMPTOMS.—Johnston* states that in the acute stages of the disease, the bud, or the growing point in the center of the crown, is affected by a vile-smelling soft rot which destroys all the younger tissues. Most of the nuts fall, the lower leaves turn yellow and the middle folded and undeveloped leaves die and hang down between the still green surrounding ones. The rot gradually spreads from the base of one spike to another until all are involved and shed their nuts; the leaf stalks become so rotten at their bases that they are no longer able to maintain their natural position and droop or else fall off. From a central diseased bud, the infection may spread downward and into the trunk of the tree for a short distance, rotting out the fundamental tissues and leaving only the fibers which are too hard to be disintegrated.

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* Johnston, John R., "The History and Cause of Cocoanut Bud-rot," Bull. 228, Bur. Plant Ind., U. S. Dept. Agr., 1912.

It has been estimated that in some cocoanut groves from 75 to 90 per cent of the trees have been destroyed by the rot.

Causal Organism.—*B. coli* (Escherich) Migula.

Method of Infection.—It is believed that the causal organism enters the host through insect bites or other mechanical injuries to the soft tissue. Insects, birds or some form of animal life are held responsible for spreading the trouble.

Control.—The removal of the diseased parts of a tree as well as spraying have proved of no benefit in controlling the disease. "The absolute destruction of diseased trees, a careful watch for the newly infected cases, and their immediate removal has done much to prevent greater loss in the various regions."

Brown Rot, A Leaf-disease of Tropical Orchids

Bacillus cypripedii—S. Hori

History and Distribution.—The brown rot of orchids was first observed by Hori* in 1906 on orchids growing in the greenhouses in Tokyo, Japan. Since then the disease has been noted on orchids from Formosa grown in their natural habitat out of doors. In 1898 v. Peglion† described a similar trouble in Italy which may be identical with the above.

Symptoms.—The rot is characterized by dirty cinnamon or light umber colored, depressed spots on the leaf-blade; these become darker with age and may increase in size so rapidly that the entire green leaf is discolored (yellowish) in a few days and dies. The rotting also spreads downward into the stem, and if the diseased leaves are not removed early, the entire stalk will be destroyed.

Causal Organism.—*Bacillus cypripedii* is a medium-sized rod with rounded ends; single or in short chains; measures 1.5 to $2\mu \times 0.5$ to 0.7μ; stains readily with aniline dyes; Gram-positive; motile by 4 peritrichate flagella; non-spore forming; smooth, light grayish white colony, with pearl luster on agar; dirty cream colony on potato; surface film on bouillon; liquefies gelatin rapidly; coagulates milk; ferments glucose with production of H and CO_2 in the ratio 1 : 3; indol positive after forty days; methylene blue reduced; ammonia and H_2S produced from bouillon; enzymes: amylase, oxidase, peroxidase; facultative anaerobe.

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*Hori, S., "A Bacterial Leaf-disease of Tropical Orchids," Cent. f. Bakt., Abt. II, Bd. 31, p. 85, 1911.

† v. Peglion, "Bacteriosi delle folie di Oncidium spec," Cent. f. Bakt., Abt. II, Bd. 5, p. 33, 1899.

PATHOGENESIS.—Pathogenic to orchids grown in the hothouse and also in their habitat.

METHOD OF INFECTION.—The germs enter the leaf tissue chiefly through wounds caused by careless washing.

CONTROL.—Use only a soft sponge soaked in a 1 : 1000 solution of mercuric chloride for wiping the leaves, and avoid excessive watering as this favors the disease.

ROT OF CAULIFLOWER AND ALLIED PLANTS

Bacillus oleraceæ—Harrison

HISTORY AND SYMPTOMS.—This rot of cauliflower and allied plants was first reported in 1901 from truck gardens in the vicinity of Guelph, Ontario. It is characterized by a soft rot of the roots and a blackening of the stems and leaves. Harrison* has found this condition to be traceable to an actively motile bacillus which invades the intercellular spaces of the plant and destroys the middle lamellæ.

CAUSAL ORGANISM.—*Bacillus oleraceæ*—Harrison is a rod with rounded ends; occurs single or in short chains; measures $2 \times 0.6\mu$; motile by means of 7 to 13 peritrichate flagella; stains with the ordinary aniline dyes; Gram-negative; in broth heavy turbidity and sediment, no pellicle; stratiform liquefaction of gelatin; on agar spreading, thin, whitish, moist, slightly opalescent; neutral red agar no change in color; litmus milk coagulated, soft curd slowly peptonized; blood serum slightly liquefied; growth positive in Uschinsky's and Fermi's solutions; potato waxy, straw-colored to moist, shining; opt. temp. 30°, max. 42°, min. 5°; thermal death-point 55°; facultative anaerobe; slight reduction of nitrates; indol slight; H_2S positive; slight gas from glucose and lactose, none from saccharose; acid from sugars; enzymes: proteolytic, diastase, cytase (pectinase).

METHOD OF INFECTION.—Infection takes place chiefly through wounds due either to mechanical or insect injuries. Warm weather combined with excessive moisture appears to favor the spread of the disease.

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* Harrison, F. C., "A Bacterial Disease of Cauliflower (*Brassica oleracea*) and Allied Plants," Cent. f. Bakt., Abt. II, Bd. 13, pp. 46, 185, 1904.

PATHOGENESIS.—Pathogenic for cauliflower, cabbage, and turnips; a soft rot can be produced in a large variety of vegetables under laboratory conditions by pure culture inoculations.

CONTROL.—Complete destruction of diseased crops by burning and crop rotation are to be recommended.

Harding and Morse,* from their extensive comparative studies of microörganisms producing soft rots of vegetables make *Bacillus oleraceæ* of Harrison identical with *B. carotovorus* of Jones.

SOFT ROT OF CALLA LILY

Bacillus aroideæ—Townsend†

A soft rot of the calla lily, distinct from other soft rots, is scattered over the calla-growing sections of the United States. The disease starts at the top of the corm and causes a rotting of the plant at or just below the surface of the ground. As a result the leaves and flower stalk turn brown and fall over. The healthy corms are white, but the infected ones are brown, soft and watery.

It is believed that the causal organism lives in the soil and enters the plants through wounds. The disease is undoubtedly spread from one locality to another by shipping slightly diseased corms.

As a means of control, only sound corms should be used, and the soil in the calla beds should be changed every three to four years.

SOFT ROT OF CARROT AND OTHER VEGETABLES

Bacillus carotovorus—Jones

A number of the cultivated plants of the north temperate zone, notably those grown for their root crops, suffer, at times, from a bacterial rot caused by a liquefying bacillus. Although probably as widely distributed as any microörganism parasitic upon plants, it was not described until 1901.‡

Bacillus carotovorus is a wound parasite which invades the intercellular spaces, dissolving the middle lamellæ and portions of the inner lamellæ, thereby establishing a condition which is known as a soft rot. Jones* has shown this solution to be due to a bacterial enzyme which he has named *pectinase*.

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* See footnote, p. 985.

† Townsend, C. O., Bull. 60, Bur. Plant Ind., U. S. Dept. Agr., 1904.

‡ Jones, L. R., "A Soft Rot of Carrot and Other Vegetables," 13th Report Vermont Exp. Station, p. 299, 1901.

CAUSAL ORGANISM.—The organism is a variable rod, majority 2.0μ by 0.8μ, rounded ends, motile by 2 to 10 peritrichate flagella; no endospores; no capsules; slight pseudozoöglœæ. Stains readily with aqueous stains. Gram-negative.

On agar, growth abundant, filiform to spreading, glistening, smooth, white, opaque to opalescent. Potato—glistening, white, decided odor, smooth, butyrous, medium grayed. Gelatin stab—filiform, liquefaction crateriform to infundibuliform, liquefaction begins second day and complete in six days. Broth—thin pellicle, clouding, abundant sediment. Milk—coagulated, slowly peptonized, rendered acid, litmus reduced. Cohn's solution—no growth. Uschinsky's solution—abundant growth. Quick tests; soft rot of uncooked carrots, turnips, cabbages. Slight gas produced from dextrose, lactose, saccharose, but not glycerin. Acid from dextrose, lactose, saccharose and glycerin. Nitrates reduced. Slight indol. Thermal death-point, 48° to 50°; grows at 37°. Optimum temperature 25° to 30°. Pathogenic to the roots of carrot, turnip, rutabaga, radish, salsify, parsnip, bulb of onion, leaf stalk of celery, leaves and scapes of hyacinth, cabbage, cauliflower, lettuce, Irish potato, fruit of tomato, eggplant and pepper.

B. oleraceæ Harrison, and *B. omnivorus* van Hall, formerly described as bacterial species capable of producing soft rots, have been reported by Harding and Morse† as identical with *B. carotovorus* and therefore to be recognized no longer as distinct species.

CONTROL.—Jones believes that the soft rots can be practically held in check by rotation of crops; by not using manure into which garden refuse has been thrown; by drying the surface of the roots thoroughly and exposing them to bright sunshine before storage; by maintaining a constant low temperature (4°) during storage.

SOFT ROT OF HYACINTH

Bacillus hyacinthi septicus—Heinz‡

A very active soft rot of the hyacinth bulb, producing a bad smelling, slimy condition in a few days, has been described by Heinz as caused by an unpigmented, motile bacillus.

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* Jones, L. R., "Pectinase, the cytolytic enzyme produced by *Bacillus carotovorus* and certain other soft rot organisms." Tech. Bull. 11, New York Agr. Exp. Sta., 1909.

† Harding and Morse, Tech. Bull. 11, New York Exp. Sta., 1909.

‡ Heinz, Cent. f. Bakt., 5, p. 535, 1899.

SOFT ROT OF MUSKMELON

Bacillus melonis—Giddings

HISTORY.—Toward the close of the season of 1907 the muskmelons in certain sections of Vermont were attacked by a soft rot. An investigation of the cause of the trouble by Giddings* showed it to be due to a microörganism which he has called *B. melonis*.

SYMPTOMS.—The decay usually begins on that part of the melon next to the soil as shown by the shrunken but generally unbroken skin over the soft diseased area. There is a complete collapse of the melons accompanied by some frothing and a disagreeable odor in the last stages. A microscopic examination of the diseased tissue, both fresh and killed, shows that the bacterial invasion is purely intercellular, and the pathological condition of the tissue manifested as a soft rot is due to the solution of the middle lamellæ.

Infection in the field appears to take place through wounds in the skin, and especially through cracks in the skin and flesh.

CAUSAL ORGANISM.—According to Giddings, *Bacillus melonis* possesses the following characteristics:

A bacillus 1.0μ to 1.7μ by 0.6μ to 0.9μ, actively motile by 4 to 6 peritrichate flagella. Endospores not produced. Gram-negative. Stains readily with aqueous stains.

In nutrient broth, strong clouding twenty-four hours, neither pellicle nor ring, slight sediment. Agar stroke, abundant, contoured, shiny, glistening, without color, opalescent growth having umbilicate elevation. Gelatin stab, infundibuliform liquefaction in two days. Cooked potato, abundant, spreading, glistening, odor of decaying potatoes. Litmus milk, coagulated and reddened in three days, no digestion. No growth in Cohn's solution. Abundant growth in Uschinsky's solution, ring, pellicle and heavy sediment, odor of hydrogen sulphide. Vegetables rotted—muskmelon, citron, carrot, potato, beet† and turnip. Growth and some acid but no gas from lactose, etc. Slight gas production from asparagin broth, abundant in fermentation tubes of milk, this gas being 99 per cent carbon dioxide. Hydrogen sulphide from nutrient broth and potato. Nitrates reduced. Slight indol. Ammonia from asparagin broth; none from broth, gelatin, milk or urea. Thermal death-point, 49° to 50°. Optimum temperature, 30°.

CONTROL.—Spraying with Bordeaux mixture or other fungicides is recommended as a preventive measure.

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* Giddings, Bull. 148, Vermont Exp. Station, 1910.

† *B. carotovorus*, Jones, associated with several soft rots, does not rot the beet.

The melons should be supported by some means to keep them from coming in direct contact with the soil, and should be supplied with adequate water during a dry season to keep them from cracking.

SOFT ROT OF THE SUGAR BEET

Bacterium teutlium—Metcalf

HISTORY.—A soft rot of the sugar beet, occurring in Nebraska, has been described by Metcalf and Hedgcock.*

SYMPTOMS.—Beets affected with the rot show the lower half badly decayed and honeycombed with "pockets" or cavities filled with a slimy, stringy fluid, colorless, sour-smelling, and alive with bacteria. The vascular bundles remain intact, while the tissue surrounding them is usually consumed. Above ground the beets appear normal.

METHOD OF INFECTION.—The germs gain entrance to the beet through wounds and abrasions in the skin, and there is good reason for believing that nematodes are responsible for many of the inoculations.

CAUSAL ORGANISM.—*Bacterium teutlium*, according to Metcalf, possesses the following characteristics:

It is a short, non-motile rod, rounded ends, 1.5μ by 0.8μ; neither capsules nor endospores have been observed; the organism stains readily with the aqueous stains. Gram-positive.

On nutrient agar, slow, scant, translucent, porcelain white, non-viscid, and penetrates the agar. On cane-sugar agar growth more rapid, viscid, watery, vitreous to translucent, colorless. Gelatin stab—scant, filiform to beaded, dirty white, no liquefaction. Cane sugar gelatin—characteristic cumulus cloud appearance in stab, no liquefaction. Nutrient broth—slight clouding and sediment, acid produced. No evidence of growth in milk. No visible growth on potato. On carrot, clear, viscid and acid. On sugar beet, viscid, clear, spreading, copious, acid, parenchyma destroyed leaving vascular tissue. No growth in Uschinsky's, Fermi's, Pasteur's, Fraenkel's, or Dunham's solution. No gas from dextrose, saccharose, etc. Facultative anaerobe. No growth at 37°. Optimum temperature, 17°. Thermal death-point, 45°.

CONTROL.—The rot is less apt to be serious if the beets are grown on relatively dry soil and if rotation of crops is practiced. The selection of resistant varieties seems to be the most practical solution of the problem.

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* Metcalf and Hedgcock, "A Soft Rot of the Sugar Beet," 17th Annual Report, Nebraska Agr. Exp. Sta., pp. 69–112, 1904.

CHAPTER V

WILTS

Wilt of Cucurbits

Bacillus tracheiphilus—Erw. Smith

History and Distribution.—The bacterial wilt of the muskmelon, cucumber, squash and pumpkin was first reported by Erwin Smith* in 1893. It is widely distributed over the United States east of the Rocky Mountains and seems to have different host preferences in different localities.

Symptoms.—The disease is characterized by a wilting of the vine, pure and simple, without any visible external cause such as mildew, rust or leaf spot. The leaves and runners wilt suddenly as if from lack of water or too hot sun, the runner becoming prostrate on the ground. From two to three days usually elapse before the wilting of the whole vine is complete, and it may remain in this wilted condition for several days, after which the leaves begin to dry up, but retain their green color for considerable time. One runner may die at a time, beginning at the tip and working back toward the root, after which a general infection is to be expected. If inoculation takes place upon the main stem, several or all of the runners may show the wilt at the same time.

The disease is caused by a bacillus whose growth fills the water ducts or tracheæ with a white, viscid material which prevents the rise of water, and wilting follows. If the severed ends of a diseased vine are rubbed together gently and separated slowly, this sticky liquid will string out in fine threads 2 to 3 cm. in length.

Method of Infection.—Under field conditions, the disease is spread principally by insects, especially the striped cucumber beetle and the common squash bug.

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* Smith, Erwin: Cent. f. Bakt., Bd. I, II., Abt., pp. 364–373, 1895.

Causal Organism.—Erwin F. Smith describes *Bacillus tracheiphilus* as a rod 1.2μ to 2.5μ by 0.5μ to 0.7μ, actively motile when young.

Growth occurs on the ordinary media. Upon agar, the growth is milk-white and extremely viscid. Upon potato, a gray film is produced, much like that of *B. typhosus;* the potato is unchanged. Gelatin is liquefied and no change occurs in milk. Acid but no gas is produced in saccharose and dextrose broths. The organism is aerobic and possibly facultatively anaerobic. Optimum temperature is between 20° and 30°. No growth at 37°. Thermal death-point, 43°.

Control.—The same precautions and preventive measures are to be recommended for the wilt of cucurbits as are given for tomato blight.

Wilt of Sweet Corn

Pseudomonas stewarti—Smith

The early varieties of sweet corn grown in the truck gardens of Long Island* are subject to a bacterial disease which manifests itself by a wilting and drying up of the leaves. It also occurs in Iowa, and it has been reported from certain parts of New Jersey.

The wilting may occur at any stage of growth, but the plants seem to be more susceptible at the time of flowering. As a rule the leaves succumb one at a time, although on the younger plants they may all wilt simultaneously. There is no external evidence which would indicate the cause of the trouble, but if a diseased stalk is cut lengthwise, the fibro-vascular bundles appear as yellow strands in the white pith. A cross-section of such a stalk will show drops of a yellow viscid substance, composed largely of bacteria, exuding from the cut ends of the bundles. The infection is not confined to the stalks but can be found in the vascular system of the leaves, husks and cobs as well. The vessels are the principal structures invaded, but in time small cavities filled with the bright yellow slime are formed in the surrounding parenchyma.

Method of Infection.—The germ may enter its host through either the roots, stomata or water pores and when once inside the vascular system, it multiplies very rapidly, fills the water tubes with a yellow slime and wilting follows.

Causal Organism.—The organism was first described by Stewart and later named *Pseudomonas stewarti* by Erwin Smith.

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* Stewart, F. C., "A Bacterial Disease of Sweet Corn." Bull. 130, N. Y. Agr. Exp. Sta., 1897.

It is a short, relatively thick, motile rod with rounded ends; occurs usually in pairs. No endospores observed. Stains readily with the aqueous stains.

It grows well upon the ordinary culture media. On agar, smooth, shining, yellowish-white to deep yellow, lobate. On potato, spreading, deep yellow becoming slightly iridescent, smooth; potato is browned. Broth—thin film, slight clouding and slight flocculent white precipitate. Milk—slight peptonization without coagulation; litmus reduced. No gas is produced from dextrose, etc. Good growth in Uschinsky's solution. Facultative anaerobe. Pathogenic for sweet corn.

CONTROL.—It is believed that the germ is disseminated on diseased seed and therefore disinfection of the seed before planting is recommended.

The disease is also spread by the use of manure which contains diseased stalks.

Varieties differ considerably in their susceptibility, and by the selection of the more resistant kinds some relief can be secured.

Rotation of crops and planting on new land, when available, should be practiced.

Field corn and pop-corn are not affected by the wilt.

WILT OF TOMATO, EGGPLANT, IRISH POTATO AND TOBACCO

Pseudomonas solanacearum—Erwin Smith

HISTORY.—A bacterial wilt affecting a number of plants of the potato family has been described by Erwin Smith.* The disease was first observed in the Atlantic coast and southern states. In 1903 Stevens† and Sackett described a wilt of tobacco in Granville County, N. C. and this, too, Smith‡ has shown to be due to the tomato wilt organism, *Ps. solanacearum*. Quite recently Miss Bryan§ has shown the same organism to be the cause of nasturtium wilt.

SYMPTOMS.—The disease usually manifests itself by a sudden wilting of the foliage, and, as a rule, with little or no yellowing. This may be indicated at first by the collapse of a single leaf, but in time the whole plant will succumb. Following the wilting, the parts affected shrivel, turn yellow, then brown, and finally black. If a diseased stem is split lengthwise, black streaks, following the fibro-vascular bundles, can be traced the whole length of the stem and often out into the corresponding leaves. The vessels are packed with bacteria which ooze out on the cut surface as little drops of a dirty white, slightly viscid liquid. The bacillus destroys the parenchyma of the pith and bark and mechanically plugs the water tubes so that the water supply from the soil is shut off and wilting follows. In the tubers of the potato, the rot begins in the blackened vascular ring and spreads in all directions, producing well-defined cavities next to the ring.

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* Smith, Erwin F., "A Bacterial Disease of the Tomato, Eggplant and Irish Potato," Bull. 12, U. S. Dept. Agr., Div. Veg. Phys. and Path., 1896.

† Stevens and Sackett, "Granville Tobacco Wilt," Bull. 188, N. Car. Exp. Sta., 1903.

‡ "Granville Tobacco Wilt," Bull. 141, U. S. Dept. Agr., Bur. Plant Industry, 1908.

§ Bryan, Mary K., "A Nasturtium Wilt Caused by *Bact. Solanacearum*," Jour. of Agr. Research, Vol. IV, No. 5, p. 451, 1915.

METHOD OF INFECTION.—Insect enemies are largely responsible for the spread of the wilt, especially above ground, while beneath the surface inoculated soil enters the roots through wounds made either by transplanting, cultivating, or nematodes. In the case of the nasturtium, stomatal infections have been demonstrated.

CAUSAL ORGANISM.—According to Smith, *Pseudomonas solanacearum* is a medium-sized rod, rounded ends; 1.5μ by 0.5μ; motile by a single polar flagellum, zoöglœæ formed in liquid media; stains readily with aqueous stains.

Zoöglœæ produced at the surface in beef broth, copious dirty white sediment, reaction made alkaline. Casein of milk dissolved without precipitation and medium becomes alkaline. On nutrient agar, growth is smooth, wet shining, slightly viscid, at first dirty white becoming yellowish, then brown; agar browned. Gelatin stab—growth best at surface, pure white, smooth, wet shining, no liquefaction or very feeble after six weeks. Potato—wet shining, not wrinkled, copious, dirty white and later brown to black; medium browned. Neither acid nor gas produced in any of the culture media or from glucose, etc. Obligate aerobe; ammonia produced in nutrient broth and potato tubes; pigment formation aided by glucose, fructose and saccharose. Grows well at 37°. Thermal death-point, 52°.

PATHOGENESIS.—Pathogenic for tomato, potato, eggplant, tobacco, Jamestown weed, black nightshade, physilis, petunia and nasturtium.

CONTROL.—If the disease is not too general, it is possible to control its spread by removing the dead plants and burning them; the early and complete destruction of all insect pests is important; if available and practical, new land or land which has not been planted to any of the potato family for a period of years, should be used; only those seeds and tubers which have come from plants grown in localities free from the disease should be planted; the use of infected manure or soil should be avoided.

Additional Bacterial Diseases

Angular Leaf Spot of Cotton, *Pseudomonas malvacearum* Smith.*
Gum Disease of Sugar Cane, *Pseudomonas vascularum* Cobb,† Smith.‡
Leaf Spot of Broom Corn, Burrill.§
Bacteriosis of Tomatoes, *Bacillus briosii* Pavarino.‖
Wilt of Banana and Plantins, *Bacillus musæ* Rorer.**
Bacteriosis of Ixia maculata, *Bacillus ixiæ* Severini.††
Bacteriosis of *Gladiolus colvilli*, *Pseudomonas gladioli* Severini.††
Bacteriosis of Orchard Grass, *Bacterium rathayi* Smith.‡‡
Rot of Potatoes, *Bacillus solanisaprus* Harrison.§§
A Bacterial Disease of the Mango, *B. mangiferæ.* Doidge.‖‖

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* Smith, Erw., Bacteria in Relation to Plant Diseases, I, p. 95, 126.

† Cobb, N. A., Rept. New So. Wales Dept. Agr., 1893, pp. 1–21.

‡ Smith, Erw., Cent. f. Bakt., II Abt., Bd. XIII, 22–23, pp. 726–729, 1904.

§ Burrill, Bull. 6, Ill. Exp. Sta., pp. 165–176, 1889. Smith and Hedges, Science, N. S., Vol. XXI, 535, p. 502, 1905.

‖ Pavarino, G. L., Atti R. Accad. Lincei. Rend. Cl. Sci. Fis., Mat. e Nat., 5, ser., 20 (1911) I, No. 5, pp. 355–358.

** Rorer, J. B., Phytopathology, I, (1911), No. 2, pp. 45–49.

†† Severini, G., Ann. Bot. (Rome), 11 (1913), No. 3, pp. 413–424.

‡‡ Smith, Erw. F., "A New Type of Bacterial Disease." Science, N. S., Vol. XXXVIII, No. 991, p. 926, 1913; Sitz. Ber. Weiner Akad., 1 Abt., Bd. CVIII, p. 597.

§§ Harrison, F. C., "A Bacterial Rot of the Potato Caused by *Bacillus solanisaprus*." Cent. f. Bakt., Abt. II, Bd. 17, p. 34, 1907.

‖‖ Doidge, Ethel M., Annals of Applied Biology, Vol. II, No. 1, May, 1915, pp. 1–45.

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